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The nuclear 1980s

Two events coming up in 1980 will influence the world's nuclear future for many years. In the spring the International Nuclear Cycle Fuel Cycle Evaluation (INFCE) will issue a report that is the result of nearly two years of study by eight working groups. Then a few months later, upwards of one hundred nations — signatories to the Non-Proliferation Treaty (NPT) — will convene for their second five-yearly review of the treaty. The events are by no means unrelated. They both reflect efforts in the world community to come to grips with the problem of capitalising on the peaceful benefits of nuclear power without opening the door to nuclear weapons. And neither, in their different ways, will be able to hold out much hope that there is any simple way forward, either in terms of technological fixes or new institutions, which will make the 1980's a time when nuclear weapons stop spreading into yet more hands.

INFCE, the backwash to President Carter's 1977 initiatives to arrest proliferation by unilateral and widely disliked measures, is likely to deliver a fairly comfortable message to nuclear states — not great surprise given the representation of nuclear enthusiasts on the working parties and the absence of any brief to consider broader issues such as environment or safety. At a meeting of the British International Studies Association last week, Dr S. Warnecke indicated that the conclusions will probably run something like this: there is no geological shortage of natural uranium although there could be political problems in international sales; if there is need for some more enrichment capacity this should be confined to technologically advanced countries (such as Japan); there is wide divergence between countries on the economic sense of reprocessing (the United States failed to get any measure of consensus on the desirability of once-through cycles); the non-proliferation risks associated with fast-breeders are no greater than those of light-water reactors; and there is as yet nothing in advanced reactor concepts

which would inherently make them more proliferation-proof than current designs. In short, the path that major nuclear nations have been taking these past ten years is neither free of proliferation risks nor is it recklessly dangerous, and no other path holds out any obvious attractions of more proliferation-proof power. This is hardly going to be a surprise to the nuclear bureaucracies of the world, although it may well disappoint President Carter who at one time hoped that INFCE would provide some international agreement on positive steps that could be taken to keep the danger of proliferation out of the nuclear fuel cycle.

The message of INFCE to the NPT Review Conference will be, then, that it is difficult if not impossible for the international technological community to do much in the final analysis to prevent the spread of nuclear weapons — a nation with a strong political will to go nuclear can easily overcome such technical obstacles as are put up. This gloomy view will coincide with unencouraging news on other fronts. On Article VI of the treaty, in which nuclear-weapon states promise as their part of the bargain to work for disarmament, there is relatively little to present except an uncertain and unimpressive SALT 2, and glacial advances towards an almost meaningless (because temporary) comprehensive test ban treaty. Furthermore the last five years have seen the emergence of a different and ominous sort of proliferator — the nation which, because of heavy hints, must be assumed to be acquiring a nuclear weapons capability even though it doesn't take the final step of firing a device.

Can the NPT survive all this? In a formal sense, yes; no doubt for the next few years it can retain all its signatories, and it must never be forgotten that there are some very desirable names on the list. But as time progresses the treaty looks more and more like every other arms control measure — a limitation only on those who have no particular desire to transgress. □

... and the broadcasting 1990s

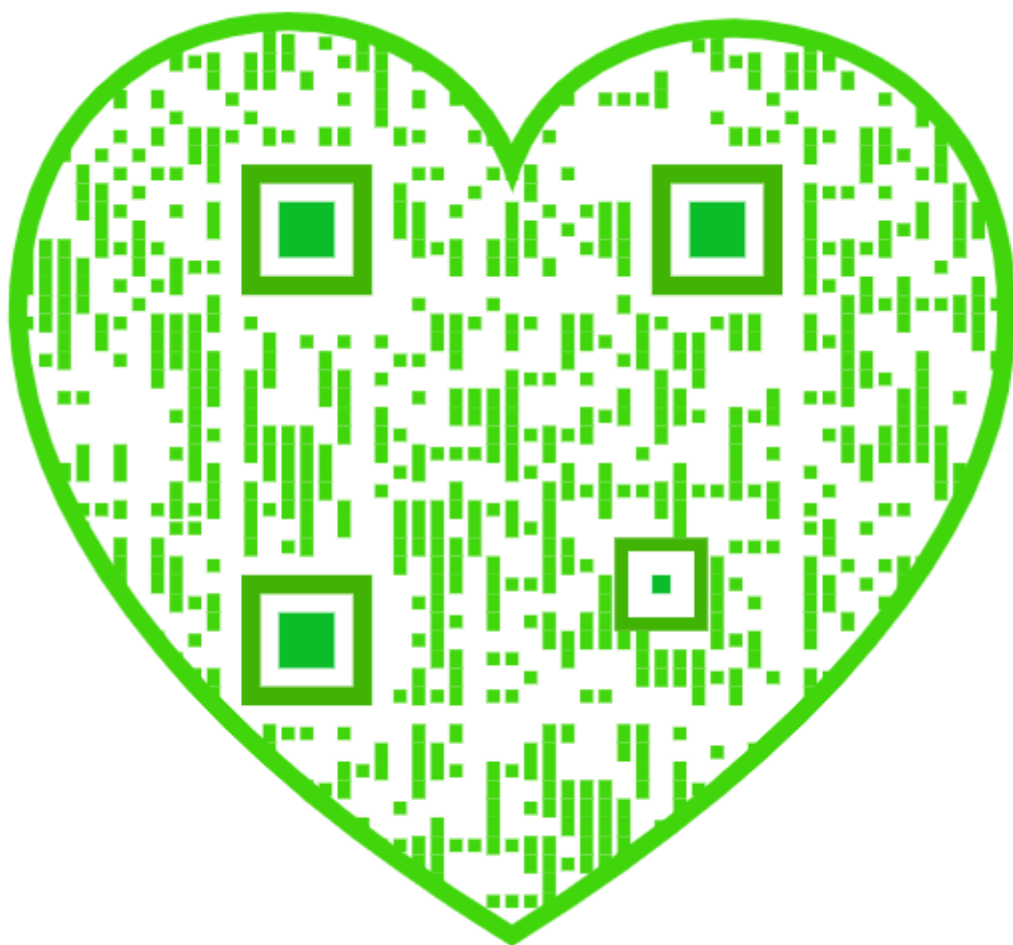
THE World Administrative Radio Conference starts this week in Geneva and is expected to run until December. Public interest is likely to focus on the allocation of frequencies, and more particularly the North-South and East-West political conflicts inherent in these allocations. But one issue which could do with much more public exposure is the question of the accessibility of television transmissions.

Radio broadcasts, with the help of the ionosphere, know no national frontiers, and as a result there is a richness of choice open to anyone with even a modest receiver. The present, ensured that it has been much more of a national affair. As a consequence access to a mass audience has been in the hands of a relatively small number of people, all with similar cultural backgrounds.

Satellite television could change all this. Within ten or fifteen years it could be possible to tune in to French, German, Italian or Russian television by directing a rooftop aerial at the appropriate satellite. The trouble is that not every country wants its people to have that freedom, which the objectors term cultural or political subversion.

As a result a 1977 conference went to great lengths to ensure, by means of satellite placings, polarisations and radiation patterns, that the cause of international understanding is not going to be given much of a boost by television.

Time is running out for brave enterprises to resist this narrow nationalism. The Geneva meeting could be the last opportunity for anyone to raise a voice against it. □



Battle heats up over control of workers' health

The US Occupational Safety and Health Administration will soon face one of the severest political challenges of its ten-year existence. In the second of three articles on the current anti-regulatory mood in Washington **David Dickson** reports on pressures on the agency and their implications for environmental research



Within the next few weeks a Senate Committee will begin discussing two bills either of which could, if implemented by Congress, significantly restrict the activities of the Occupational Safety and Health Administration (OSHA). One proposes a partial exemption for a large number of companies from the provisions of the act which set up the agency in 1970; the second is a stronger bill which would mean total exemption for small businesses removing the agency's jurisdiction over more than four million workers.

Attempts to limit the activities of OSHA — perhaps the federal agency least loved by the US business community — have frequently been launched in Congress, but have seldom gathered much momentum. This year, however, the situation of different. Defenders of the agency have less support than previously; while critics, buoyed by their success in securing an amendment to OSHA's appropriations bill which effectively bars the agency from routine safety checks on 1.5 million establishments, are confident that at least one of the new bills may reach the statue book.

Disenchantment with OSHA has been directly fuelled by the anti-regulatory mood that is increasingly dominating both the Congress and the administration. Much of this has its roots in the economic argument that excessive regulation has been an important contributor to inflation, in particular reducing the rate of technological innovation and thus slowing productivity growth.

But behind this there is a political argument over who should have prime responsibility for protecting the health of the worker. Whether it should be the federal government, in its role of social regulator; or whether the responsibility should be passed partially back to private industry, operating under broad government guidelines, but responding primarily to economic incentives and the dictates of the market-place.

Support for the latter view is growing in strength. But to the extent that such sentiments have already been relected in various Congressional actions, they have

been strongly condemned by the trade unions. In a report on recent and current legislative moves, for example, the United Steelworkers of America says that 1978 and 1979 were "dismal" years in Congress for workers safety and health.

"The errant pot shots that had been fired at OSHA every year since its enactment have now improved in their accuracy and their potency, and real erosion is beginning to take place", the union says.

The seeds of the present conflict between supporters and critics of OSHA were sown in the 1970 act establishing the agency. This requires OSHA to work towards achieving a "safe and healthful" environment for every US worker — but gave little specific indication as to which criteria should be used to make this assessment.

Initially OSHA came under steady fire for appearing to concentrate on "nit-picking" issues, such as the height of fire extinguishers above the floor, and ignoring many of the more important health and safety standards.

Its image of ineffectiveness and petty-mindedness has been successfully revamped under OSHA's present head, Dr Eula Bingham. Appointment by President Carter in 1977, Dr Bingham has shifted the main emphasis from safety to health — a move reflected in the fact that OSHA has produced more health standards in the last two years than it did in the previous six.

Agency officials point to one of its major successes as being an increased public awareness of the importance of occupational health as a social issue. But they acknowledge the paradox that this has occurred precisely at a time when there are growing demands for the government to reduce its regulatory activities; and that this puts OSHA and its supporters in a vulnerable situation.

Evidence of this shift in public mood is provided by the speed with which even "liberal" politicians are now jumping on the regulatory band-wagon. The more restrictive of the two proposed Senate bills, for example, has been submitted by Senator Franch Church of Idaho, considered a liberal in US politics, but facing a tough challenge from the right in

his re-election battle next year.

"Having someone like Church, who we do not consider an enemy, against you makes things a lot harder to deal with," says George Taylor of the AFL-CIO (American Federation of Labour — Council of Industrial Organisations), who foresees a need for the unions to "fight like hell" to protect some of the gains made in recent years.

It has become politically acceptable to challenge the activities of regulatory agencies. Speaking at a meeting of the American Chemical Society in Washington two weeks ago, for example, Dr P M Nortling, director of health and safety for the Du Pont Chemical company, said that although the number of citations for illegal practices issued by OSHA had diminished over the past two years, the proportion challenged by industry had almost doubled, from seven to 12% over this period.

Corporate attitudes

"There has been an important change in corporate attitudes. In the past, corporations were unwilling to appear obstructive on health and safety issues. That reluctance is now giving way, and we are now seeing the chemical industry taking a very active role in challenging regulatory proposals, particularly those which we feel impose economic burdens incommensurate with the expected benefits," Dr Norling said.

Ironically many of the current controversies result from a surfeit of laboratory data about the physiological effects of particular substances — at least when compared to the relative lack of epidemiological data on human health implications of the type which can be used as a solid basis for regulation.

A typical case in point is OSHA's proposal to implement "generic" carcinogen controls and standards. Under this proposal, first put forward two years ago and expected to be published in its final form next month, the agency will classify as a carcinogen any substance known to produce tumours in any single species of laboratory animal — and will demand that

Above: working in a steel foundry

occupational exposure to this substance should be reduced as low as technologically feasible.

OSHA scientists argue that, given the many uncertainties that surround the mechanisms of carcinogenesis, workers should be given the benefit of the doubt. And this means protecting them against any substance bearing the least suspicion of carcinogenic properties.

The chemical industry, on the other hand, has attacked this approach as being both unnecessarily expensive — it quotes a cost for implementing the proposals as between \$8 and \$88 million — and as inherently “unscientific”. The industry argues that more substantial data is required before taking such drastic action; this might, for example, include classifying carcinogens in terms of relative potency.

The industry has formed a lobbying group, the American Industrial Health Council, specifically to challenge OSHA's proposals — referred to last week as an “administrative short-cut” by AIHC executive director Ronald A Lang.

One AIHC proposal meeting with more sympathy in the administration, however, is that there should be a clearer distinction between the scientific and the political decisions made in the regulatory process. The AIHC has proposed that the National Academy of Sciences appoint a panel of “the best scientists the government can tap” to assess whether a particular substance is or is not a potential human hazard, and if so to what degree. The appropriate control mechanisms would then be agreed by a separate body.

A similar, although not identical, two-stage process for dealing with occupational hazards is already being developed within the administration, using the Department of Health, Education and Welfare's National Toxicology Programme to centralise the scientific assessment of potential hazards; and further developments along these lines are expected to be contained in a report from the President's newly appointed Regulatory Council, due in a few weeks time.

Even though such centralisation may have advantages in terms of efficiency, however, not everyone is totally convinced of its merits, some fearing that it might put a strait-jacket on the flexibility made possible by a plurality of programmes. “If there is an indication that these joint programmes are used to explore certain questions and to neglect others, that will be a real problem,” says Dr Robbins of the National Institute of Occupational Safety and Health.

But the pressures to change the whole shape of Washington's regulatory apparatus continue to build. Until a few months ago, the dominant demand was that this apparatus should be “rationalised” in the interests of cost effectiveness. More recently, there has been what Dr Frank Press, director of the Office of Science and Technology Policy,

has referred to as the emergence of a “new philosophy” emphasising economic incentives rather than regulatory clout.

Speaking at the ACS meeting two weeks ago, Dr Press suggested that industry should be given more flexibility in deciding how to achieve overall regulatory goals established by society. This could be done, for example, by applying the “bubble concept” used for air pollution control — requiring merely that a plant's total effluence release complies with federal standards — to health and safety.

“One of the important things to recognise regarding this approach to regulation is that it provides new incentive to industry to innovate. It takes some of the repressiveness and uncertainty out of regulation, and relies more on market-forces to achieve the desired goals in environmental quality, health and safety.”

New legislation along these lines may well emerge in the next few weeks, when President Carter announces the conclusions that he has reached following a year-long review by the Department of Commerce of the effects of government policy on industrial innovation; the forty recommendations passed to the White House for presidential decisions include a number on regulatory reform, including for example a proposal to shift from design to performance criteria as the basis for regulation.

A number of observers in Washington feel that, with an election year approaching, it would be difficult for the President to support any major roll-back in occupational safety legislation — if only because trade union support is probably more important than that of environmentalists who have complained of the impact of proposed energy legislation.

But within the administration there is considerable dispute over whether the incentives approach is the right one to take. OSHA, for example, has been strongly opposed to demands that health and safety

regulation should be justified on economic grounds, arguing in particular that there is no way that a worker's health can be adequately quantified — and certainly not by putting a market value on it.

Indeed almost all sides in the dispute on an appropriate policy now seem to accept that the vagaries of cost-benefit analysis are such as to make it an inadequate basis for regulatory decision-making — and that no statistic can really be trusted to give a full reflection of either the costs of regulation or its benefits.

As far as Congress is concerned, the response has been explicitly political. “Ultimately out belief is that the protection of the work-force is the responsibility of the employer, working with the employee, not of the OSHA policeman holding a club” says Mark de Bernardo of the Chamber of Commerce, a Washington-based group representing private industry interests which is providing active support to legislative attempts to change OSHA's mandate.

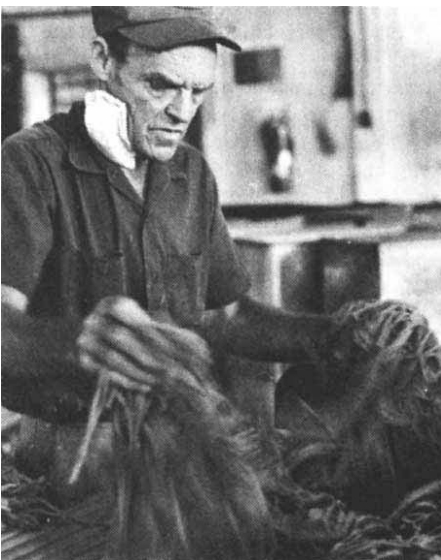
Trade unions

Trade unions and their supporters, however, see things differently. Although critical of the relative lack of impact which OSHA has had in the past, they credit it with many important gains, for example some of its recent programmes in worker education. And they are convinced that the health of workers can be better achieved through an effective regulatory agency rather than through the collective bargaining process.

“Protecting the gains made by OSHA is going to require vigilance and hard work,” says George Coling of the Urban Environment Congerence, a lobby group which has been engaged in developing a coalition of labour, environmentalist and minority groups on occupational health issues. “It is not a battle for the agency, but it is a battle for health — and the agency is the tool that we need.”

Ironically, the one aspect of federal health and safety efforts which has so far been relatively immune from Congressional attack has been the research budget. The budget for NIOSH, for example, is scheduled to increase by 25 per cent for the fiscal year 1980, reflecting the administration's commitment, articulated through the science adviser's office, to improve the “science base” of its various spheres of activity.

Yet even this may have its price. “It is possible that Congress may want to put more money into research rather than into adequate reinforcement. We obviously welcome the extra funds; but in the long-run such a shift in emphasis would not be a good thing; it might mean that we could produce more results, but it would give the administration less ability to put these results into practice,” says Dr Robbins of NIOSH. □



US textile worker: stringent limits to cotton dust exposure have been one of OSHA's recent successes

Next week: problems with non-proliferation

Scientists leaked H-bomb secrets, says US

US Government experts believe that the authors of two press stories outlining the concepts behind the hydrogen bomb may have been helped by leaks from scientists at the government's own Lawrence Livermore and Argonne national laboratories.

The government had tried to stop publication of an article by Howard Morland entitled "The H-bomb Secret — How We Got It, Why We're Telling It" in the *Progressive* magazine. However, the government dropped its case after the publication last week of a lengthy letter outlining the details of the H-bomb in a Wisconsin newspaper, the *Madison Press Connection*.

The letter, written by a computer-programmer, Charles Hansen, has roused the suspicions of government nuclear experts, who claim that Hansen used leaks from the government's secret files in its case against the *Progressive* in addition to repeating the mistakes made by Morland in his article. As for the latter, the government claimed in a brief that "The evidence suggests that Morland was able to write accurately about the H-bomb secret only because he had significant guidance by a person or persons with access to classified material".

Both authors insist that they based their articles on information available to the public in libraries, lectures, and textbooks, and that the similarities in the two articles could be explained by the fact that they used the same sources.

Despite the decision to drop the *Progressive* case, the legal battles are not over, since the government has now asked the Justice Department to determine whether there is a case for criminal prosecution under the Atomic Energy Act.

But lawyers on both sides have doubts as to whether a firm case could be made. The lawyers for the *Progressive* argue that the Act is too loosely defined for a successful prosecution; and some lawyers in the Justice Department believe the government's case would be fundamentally flawed, as the many of the "secrets" are already in the public domain.

Anne Norman

Poland seeks cooperation on nutrition

Fundamental research into human nutrition, including data gathering and the design of experiments suitable for use on human subjects, should become a major area of international cooperation, Professor Kazimierz Szebiotko of the Poznan Agricultural Academy told *Nature*

last week. He was attending a UK/Polish symposium on food technology at the National College of Food Technology, Weybridge.

Poland already has a well-developed programme of food research. Protein production and its optimum use rates as a "governmental problem" in the Polish hierarchy of research and development, "commanding the highest priority. The eight-point programme ranges from plant genetics to unconventional protein production, and from the biochemistry of aminoacids to the optimum animal feeds.

So far, said Professor Szebiotko, results have been generally better than expected. A soya-bean has been developed with the shorter growing period necessary for Poland, and already some 500 ha have been grown successfully. In collaboration with a Canadian team, a strain of rape-seed has been developed with very low content of erucic acid (suspected of being a carcinogen).

There have, however, been setbacks. A project for processing inconveniently small fish and fish scraps into edible form turned out well — but ran into trouble when fish stocks declined. There are certain logistic difficulties; in his own field of single-cell protein production from agricultural wastes, Professor Szebiotko said, developing the process was fairly straight forward. What was needed now was research into the "economics of cooperation with industry".

This did not seem to have been the case, incidentally, with TVP. According to Professor Jan Zaleski of the Ministry of Food Production, who led the delegation, for some years now all Polish hamburgers, mince and many kinds of sausages have contained TVP "extender" (20% in the case of hamburgers and about 12% for sausages), the new recipe being quietly introduced and the non-extended product simultaneously phased out by agreement between the Ministries of Food Production and Health — without any indication to the public. This, incidentally, was not the best procedure in Professor Szebiotko's opinion. He would have preferred the open launching of frankly soya lines.

In the future, he hoped, genetic engineering would form a fruitful field of international cooperation. Other such "themes for the future", to be tackled at the international level, he suggested, might include the biochemistry of nitrogen fixation, the optimal use of water resources (also a major concern in Poland), and his own particular interest, optimal nutrition.

So little is known about this, he stressed, while so much work has been done on malnutrition and obesity. What is needed, he suggested, is a data-bank of nutritional experience collected over the whole world. No one country, or even group of countries, could undertake this. It is a task for the United Nations agencies, for WHO and FAO.

Vera Rich

Cubans to join the space race before 1983

Cuba's future cosmonauts have received special training and acclimatization to enable them to cope with a possible landing in the Siberian winter, the chief training officer of the Interkosmos programme, former cosmonaut V. A. Shalato, told a Moscow press-conference recently, despite the fact that Soviet space planners are usually reluctant to give any details of future plans or cosmonaut training.

The press conference was, formally, a winding up of the latest series of Salyut-6 missions. The emphasis on future Cuban participation does not necessarily indicate an imminent Cuban launch. It could simply be a response to the Havana non-aligned conference and the large Latin-american presence at the press-conference.

Shalato would not commit himself to a date for the Cuban launch, recalling only that it was originally announced that all the Comecon allies would have had a cosmonaut in orbit by 1983, and that "we have every reason to think we will meet this date, or perhaps it will be a little earlier." He did, however, say that the Cubans had completed their general theoretical work and had now moved on to crew training.

At present, the two candidates are officially on holiday in Cuba, (where incidentally, Interkosmos maintains a cosmonauts' rest home). One of them has also been taking examinations at Havana University.

Meanwhile, according to Academician Boris Petrov, Chairman of the Interkosmos council, the programme of on-board-experiments proposed by the Cuban scientists for the future cosmonauts to perform is undergoing expert scrutiny by "specialists of both countries".

Vera Rich



"If they're gonna put a Cuban in space maybe we can fix it to be Fidel Castro!"

Lake Balaton: going green

HUNGARY's Plant Protection and Agrochemical Service was originally established 25 years ago to boost agricultural production by the proper application of modern fertilizers and pesticides, a task which it carries out efficiently and well from its 20 sophisticated research centres (one in each county and one in Budapest). During the last three years, however, the centres in Zala, Veszprem and Somogy counties have taken on an additional role, the protection of the water quality of Lake Balaton. This is a task of some urgency: a recent report to the Hungarian government stated that with the present rate of chemical input, and no preventive measures, the majority of the lake will be green with eutrophication and unacceptable for leisure use within 15 years.

Lake Balaton is the largest body of fresh water in continental Europe with an area at mean water level of 595 km², and it is Hungary's main holiday area. In 1963, a special government decree pronounced that the region should be developed primarily to meet the needs of domestic and foreign tourism. A number of highrise hotels sprang up along the lake shore — not always with sufficient attention to effluent disposal.

The main problem, however, is agriculture in the catchment basin, an area of some 5,180 km², excluding the lake surface. The main crops are wheat, maize, rape and, on the northern shore, grapes. Modernisation of agriculture has led to a considerable increase in the use of agricultural chemicals. By 1975, the average concentration of active nitrogen phosphorus and potassium substances (NPK) had reached 400 kg/ha in Somogy county, 330 in Veszprem, and 380 in Zala. The daily discharge into the lake via the river Zala and other feeders was 764 kg of total phosphorus and 1,600 kg of total nitrogen from plant nutrients, with an additional 254 kg of total phosphorus and 533 kg of total nitrogen from treated effluent.

To exacerbate the problem, environmental protection in Hungary was covered by a piecemeal series of laws until as recently as 1976. These were often outdated, as, for example, a 1947 order of the Ministry of the Interior which made it an offence to encourage draught animals by shouting. In 1976, however, a new and comprehensive act came into force: "On the protection of the human environment". To implement this legislation, the former Office of the Environment was upgraded and given independent status as the State Council for the Environment and Nature Conservation, with a secretary of state, Dr Gyorgy Gonda, at its head. To deal with the specific problem of the Balaton area, a Coordinating Council for Research on

Lake Balaton was established under the auspices of the Academy of Sciences, headed by Dr Istvan Lang, Deputy General Secretary of the Academy.

A complex ecological research programme was inaugurated, originally with some 200 subjects on the agenda. After what Dr Lang describes as "repeated professional consultations" these were finally whittled down to three priority research areas: factors characterising, impairing or improving water quality in the lake; the social sciences background to environmental decisions in the region; and the environmental factors affecting recreation and tourism. In addition, two longer-term studies were also given priority status: the review and synthesis of the principal scientific results and professional experience relating to the region, and the working out of the scientific basis for an integrated monitoring system.

Some 40 research institutes, university departments and industrial research laboratories were drawn into the project. Government guidelines indicated that the lake water from now on must be used for drinking and recreation only; even treated effluent is to be excluded. While civil engineers and architects are busy on a crash programme of sewerage development, the three plant protection stations in the counties bordering the lake have joined forces with the National Water Authority to monitor and prevent the run-off of chemicals.

Close to the lake and its tributaries, an upper limit of 160 kg/ha of active NPK has been set, with some pesticides and fertilizers forbidden completely. The restricted zone is normally 1 km wide, measured from the bank, with very severe restrictions south of highway 71 on the north shore of the lake. Although a reduction in fertilizer use should lead to decreased crop yield, recent rises in the price of fertilizers have raised the general question of how cost-effective it is to apply massive doses of fertilizer. Many of the erstwhile lavish cooperatives are in any case settling for slightly lower yields. One of the main research subjects for the Plant Protection and Agrochemical Service is how to increase crop-yield without increasing the amount of fertilizer used. As far as pesticides are concerned, the Plant Protection and Agrochemical Service was already operating a compulsory soil and residue monitoring service for food crops. In the three lake counties, this was extended, in 1976, to the monitoring of pesticide run-offs. After an initial survey carried out together with the National Water Authority, a set of soil and water sampling sites were chosen at random positions. These sites are monitored every fortnight or month (according to agricultural conditions). According to Dr Sandor Bordas of the



Balaton fisherman mends his nets

Budapest Plant Protection and Agrochemical Station, it is now possible, on the basis of the last three years' results "to follow the dynamics of pollution, and to have a regular view of the quality of water and soil in relation to pesticides".

According to the research team at the Zalaegerszeg Plant Protection and Agrochemical Station, the new restrictions will have little overall effect on the region's agriculture. With some crops, however, there may be "difficulties". Vine-growing, in particular, is now very difficult in the restricted zone of the north shore, and the biological research institute at Szeged has started research into disease-resistant strains, which would reduce the need for pesticides. So far, however, no variety has been obtained that is entirely resistant to *Plasmopora viticola* (downy mildew). Should vinegrowers wish to restock, they explained, the government will give direct financial assistance to state or cooperative vineyards. Much of Hungary's viticulture, however, takes place in the private sector, on the small 'household plots' which provide some 30% of all Hungary's crops. Small growers wishing to make the change-over, said the Zalaegerszeg team, get 8 years free of tax on the new vineyard.

The small grower, incidentally, is potentially one of the weak links in the whole plant protection service. On cooperative and state farms, the use of agricultural chemicals is strictly controlled by specially trained Plant Protection Engineers. No such restrictions can be imposed on the private grower — other than by limiting the sale of the most sophisticated products to the state sector. Theoretically, then, there is nothing to stop the owners of summer cottages on the north shore from pouring unacceptable concentrations of fertilizer on to their gardens — except a growing public awareness with ecological problems, which is currently being promoted by a massive campaign in the media.

Vera Rich

news in brief

Pillinger wins appeal against dismissal: A UK employment appeals tribunal has ruled that Dr David Pillinger was unfairly dismissed from his post as a research biochemist at the Christie Hospital in Withington near Manchester. Pillinger, 40, had held a permanent post at the Christie since 1962 but was made redundant when the Medical Research Council failed to fund an experimental chemotherapy project he was working on (28 January). A previous tribunal ruled against Pillinger but on appeal Mr Justice Flynne found that the redundancy was "grossly unfair" since the hospital did not make an effort to find out why Pillinger's grant was denied. The hospital will now apply to the Court of Appeal for leave to appeal and if this is granted it will present its case to the Court later in the year. In the meantime Pillinger is without compensation. Even if the Court of Appeal finds in his favour he will have to face a further tribunal to obtain remuneration for the damages caused by his dismissal.

Two hundred demonstrate for Piperno in Paris: Demonstrators marched through Paris last week in support of Franco Piperno, the Italian physicist and left-wing political activist who is threatened with extradition to Italy for "activities directed against the State". Piperno has drawn support from intellectuals in both France and Italy against his extradition. In Paris, the Comité des Intellectuels pour l'Europe des Libertés (CIEL) — an organisation of leading European intellectuals headed by playwright Eugene Ionesco — issued a statement last Thursday opposing a renewed demand for Piperno's extradition by the Italian authorities. "The rules of jurisprudence are opposed to an extradition based on manifestly political motives. The Paris court refused this extradition the first time and the Italian judge, seeing the inanity of his first formulation has prepared a second dossier consisting of an improbable judicial patchwork of no less than 46 indictments." In Italy, 50 Italian intellectuals, including writer Romaine Gary and film director Bernardo Bertolucci have expressed their opposition to the arrest of Piperno in Paris and the persecution of other activists in the Workers Autonomy Movement, an independent political grouping to the left of the Italian Communist Party. The Paris Court of Appeal will examine the second extradition request on 26 September.

Canada announces inquiry into nuclear energy: The Canadian Minister for energy, mines and resources, Mr Ray Hnatyshyn, announced last week that the Canadian government is planning an open parliamentary inquiry into "the whole field of nuclear energy". In a speech to provincial mines ministers in Winnipeg, he said that the inquiry will be conducted by a parliamentary committee, but that the government did not intend to place any type of moratorium on the further development of nuclear power during the inquiry. Full details of the nature and scope of the inquiry are to be decided after the Canadian parliament reconvenes in October.

French unions find fault with reactor part: Two French trade union federations, the CFDT representing metallurgy workers and the CGT representing workers in the energy industries, have announced "important" faults in the fabrication of a part destined for French nuclear reactor assemblies. The two federations made their announcement in a letter to the Minister for Industry, M. Andre Giraud, on 22 September. An official inquiry has been ordered into the origin and evolution of the fault. The fault in question consists of fissures from 7 to 8 millimetres long and 6 millimetres deep in the tubular plates of the heat exchanger-steam generator, one face of which has contact with the water from the primary reactor cooling circuit. The CFDT says that with some 20 reactors affected, French on-site safety measures are not adequate to deal with the situation. The CGT while saying that there is no immediate hazard is calling upon its

members not to start up the reactors without "fully guaranteed security conditions." A decision will be made at the end of the month whether to start up certain reactors or not.

US nuclear fuel factory shut down: The director of the Nuclear Regulatory Commission's safety division has ordered the shut-down of a factory that manufactures fuel for US Navy nuclear submarines. The factory was closed after an NRC inventory revealed that a significant amount of highly enriched uranium was unaccounted for. The exact amount has not been disclosed but regulations stipulate a shutdown within 72 hours if more than 19.8 pounds of uranium is missing. The Nuclear Fuels Services Inc. facility which is located in Erwin, Tennessee has had a history of problems. In April 1977, the factory was fined \$53,000 by the NRC for failure to provide adequate security services. The facility will remain closed for 45 days while a complete uranium inventory is made.

US Navy tested germ warfare on San Francisco: The US Navy blanketed 117 square miles of San Francisco with an aerosol spray of bacteria in 1950 in an attempt to measure the effects of biological warfare, according to recently released military records. For six days in September 1950, a Navy ship released an aerosol containing *Serratia* sp. bacterial (thought to be harmless at the time) over the city. In spite of a subsequent outbreak of *Serratia*-related pneumonia the Navy kept quiet about the tests until last week when documents were released to the family of Edward Nevins, a victim who died during the outbreak. The family is suing the Army for \$11 million. The Nevins case raises questions about 239 similar open-air experiments conducted by the Army in the twenty years between 1949 and 1969.

Telecommunications for Third World: The use of new technology to provide reliable telecommunications for remote rural areas in Third World countries was one of the few practical suggestions to be made at the recent UNCSTD meeting in Vienna. Already worked out in considerable detail and coordinated by the International Telecommunications Union in Geneva, the proposed Global Domestic Satellite System aims to make use of the increasing reliability and convenience of geostationary satellites (types such as INTELSAT 5 and SYNCOM 4 were referred to in the paper) as the central transmission facility. Such a satellite would transmit to transistorised ground stations operable on a 12-volt battery pack costing an estimated \$ US 15,000. Retransmission would be by line of sight radio to the surrounding area. ITU believe that the rapid growth and refinement of the technology involved indicates the feasibility of launchings in 1982/3.

India to train Vietnamese scientists: India will train scientists from Socialist Republic of Vietnam under a programme of science and technology co-operation signed in New Delhi in July. The training will be in survey techniques, railway technology, information sciences, water resource management and research in drugs and medicinal plants. Areas of further co-operation between the two countries, in the fields of water management, pollution control, highway engineering research, traditional herbal remedies and medicinal plants will be explored by Indian scientists who will be sent to Vietnam. The programme is follow-up action of an agreement on cooperation between the two countries signed in Vietnam earlier. *Zaka Imam, New Delhi.*

Thai researchers go for mushrooms, not poppies: The Thailand Institute of Scientific and Technological Research, Bangkok, has been investigating the cultivation of the button mushroom on paddy straw, one of the major agricultural wastes of the country. It is hoped that it will be suitable for cultivation by the poor hill tribes of North Thailand — where the opium poppy presently provides much income.

Why Britain does not need a Minister for Science

By Margaret Thatcher

Ian Lloyd, a Conservative MP, was the chairman of the sub-committee on science of the Commons Select Committee on Science and Technology. We publish excerpts from an exchange between him and Margaret Thatcher on science policy

Dear Prime Minister, I hope you will not mind my sending you two documents which bear on the exchange of views on science policy administration between us at Question Time on Thursday 12 July. The first is an article by Judy Redfearn in *Nature* (12 July, 98-99) on the role now being filled by Pierre Aigrain in France. The second is a guest leader in *New Scientist* which I have been asked to write (2 August, 346).

Pierre Aigrain was Chairman of the Committee on Information Technology in the EEC which now bears his name. He has made a distinguished and forceful contribution to science policy. The role which he now discharges in the French government is precisely what I had in mind when putting my question. Apart from yourself, no Minister, to my knowledge, has the same function here. There is no national "science budget", only a series of departmental science budgets... There is, therefore, no overall view and such a view is urgently required.

Aigrain has commissioned and is publishing a report on the organisation of French science and the resource allocation policy behind it which he claims, I believe rightly, to be unique. I do not know of any report which is as comprehensive as "L'état des sciences et des techniques françaises" and I am not aware of any document in the UK which provides information from which government, or anyone else interested in science policy, could draw conclusions similar to those set out in the inset article on page 99 of *Nature*.

I hold a strong conviction that we need such a Minister, such an allocation of responsibility, and that a similar report on the organisation and effectiveness of scientific research within the UK is required. I do not believe that a general sympathy for the importance of science and technology within the existing departmental structure — important though it may be — is a sufficient condition for the successful application of science in the United Kingdom in today's circumstances...

My other reason for raising this issue is the profound dismay which I believe we have caused by a failure to re-establish the Select Committee on Science and Technology. I know that the Parliamentary and Scientific Committee is most concerned about this and that this lies behind the decision of the House of Lords to set up their own Select Committee on

Science and Technology which they hope eventually to broaden into a joint committee of both Houses. While this is no substitute for the Commons committee, it will salvage something from the wreck and I strongly support their Lordship's initiative. Their contribution of scientific expertise will be most important and it is conceivable that such a joint committee, provided it has the government's support, may well develop into an organisation around which a thorough and wide ranging review of national policy and performance in the field of science and technology policy might be built...

Dear Ian, You will remember that your point was extensively debated here in the early 1970s. In fact there was then a more centralised science budget than there is now... But the centralised system was not considered satisfactory.

Our system was given its present shape by the 1972 White Paper "Framework for Government Research and Development" (Cmnd 5046)... These arrangements were reviewed by the last Government. The result of the review was published in March this year as a White Paper (Cmnd 7499).

Our system recognised that Government-sponsored applied science and technology is not an end in itself, but a means of helping to achieve the Government's policies and objectives. It follows that policy on applied science and technology in any sector should be associated with policy on investment, human resources, market needs and other factors, and should therefore be the concern of the Minister responsible for overall policy in that sector. But there is one sector — fundamental research — where there is no close link between research and policy. For that, it makes sense to entrust responsibility to the Minister who is responsible for those institutions of higher education where much of this type of research is done...

If we went over to a centralised system with a separate Minister for Science with his own department we would have to accept the disadvantage of divorcing those responsible for applied R and D from those concerned with formulating and implementing the policies to which their R and D related. In fundamental science we would have an unwelcome division between responsibility for higher education and for the scientific community supported by the Research Councils.

At present we have machinery to ensure



Sorry about the Select Committee

that there is no harmful overlap between Departmental R and D programmes and policies, that no gaps arise, that policy questions with a major scientific or technological content are considered interdepartmentally; where this is necessary and that the quality and direction of R and D in any area, or over all areas, can be assessed. Since 1976 a committee of permanent secretaries and chief scientists has provided interdepartmental coordination of science and technology matters at high official level; and the Central Policy Review Staff play an active part in the overview aspect...

I note your view that we need for Britain a report on the organisation of our science and the resources that go into it on the lines of what the French are doing. I should like to consider in due course whether such a report would be valuable... The Research Councils and several Departments publish annual reports on the R and D, and it may take some time to digest the implications of the reduction in public expenditure to which we are committed. But I am quite sure that we do not need a Minister for Science to prepare such a report...

I am not saying that our present system should never be adapted. On the contrary, I intend to keep an eye on how it is and modify it as may be required. I recognised, for example, that under the present arrangements issues may arise which straddle the responsibility of several Ministers to such an extent that it would not be sensible to ask one of them to take the lead. In such a case I would myself play a coordinating role...

Personally I was very sorry that the House decided not to re-establish the former Select Committee on Science and Technology. I thought it did valuable work and was complementary to the role of departments. But you and I were overruled by the vote — however much we both regret it.

From begging bowl to bread basket

In his final report on science in India, **Anil Agarwal** describes the green revolution that has put India in the position of being able to feed its starving millions. But only in principle . . .

In 1967, William and Paul Paddock wrote a book called "Famine 1975 — America's Decision: Who Will Survive?" In this book, the Paddock brothers classified developing countries, based on their population growth rates and potential to grow food, into three categories: 'can't be saved', 'walking wounded' and 'should receive food'. India suffered the ignominy of not only being classified as 'can't be saved' but also of being accredited with the comment that it "is the bellwether that shows the path which the others, like sheep going to slaughter, are following." India was thus a country, according to these authors, to which the US should not have bothered either to provide aid or food.

It is therefore almost with glee that Dr M.S. Swaminathan, the former Director-General of the Indian Council of Agricultural Research (ICAR), seems to have quoted these statements of the Paddock brothers in his lecture to the Administrative Staff College of India at Hyderabad now entitled: "From Begging Bowl to Bread Basket". For indeed, as seen in 1979, the genetic package that India adopted in the late 1960s in the name of the green revolution, is now increasingly turning it into a major, and hopefully stable, producer of cereals. India has had bumper harvests for the last four years (1975-79). In 1978-79, the grain output touched a high of 126 million tonnes, culminating in a record government-held grain reserve of 26 million tonnes, which is more than half the total import of foodgrains by the Third World in 1975.

India has recently supplied several countries like Afghanistan, Bangladesh, Viet-Nam and USSR with food shipments and, at one point, even Pakistan, was reported to be considering a request to India for grain. A special committee appointed by the government recently recommended that India should start planning grain and vegetable exports on a regular basis to develop stable markets, though initially, it said, the grain exports could be kept at a low level of 1 million tonne a year. Some Indians still remain cautious about rushing into a venture that may not be possible to sustain. Many indeed find it difficult to believe that India can become a grain exporter. Without any doubt, the succession of good weather for four years has largely been responsible for the bumper harvests, and India's climatic pattern shows that after every 3-4 years of good weather, there is a recurrence of 1-2

years of serious drought. But, simultaneously, a feeling is growing that with the spread of irrigation, fertiliser use and high acreage under high yielding varieties (HYVs), the increases in agricultural output that India has been witnessing, are of a permanent nature.

In fact, the time when this belief will be tested is already here. Northern India is currently witnessing, according to some meteorological reports, its longest dry spell since 1906. The Chief Minister of the populous northern state of Uttar Pradesh recently claimed that Rs 7000 million worth of agricultural output has been destroyed in his state alone. But Dr Swaminathan, now the Agriculture Secretary, claimed at a press conference that the fall in India's total production will certainly not be as precipitous as it used to be after drought years in the past. And, furthermore, he pointed out that the problem in 1979 is not one of grain scarcity, but one of organising efficient distribution of grains and launching crash food-for-work programmes so that the rural poor, whose crop has been destroyed, can get employment.

Even outside India, several organisations are waking up to the agricultural potential of India. The World Bank, in its annual report for 1979, released this week, claims that there are growing indications that the 'floor level' of India's grain output has been raised. James Grant, President of the Overseas Development Council, Washington, even believes that India is fast acquiring the potential not only to feed itself but also countries outside. Crop yields in India are still very low. Its acreage is very large. This gives India an enormous 'untapped yield reservoir', as agricultural scientists call it.

How to triple output of grain

In an article published in a book entitled *Mobilising Technology for Development*, released last month at the UNCSTD, Grant explains: "Yields per acre in India (particularly of rice, which has the largest acreage) are approximately 1,000 pounds, compared to 3000-4000 pounds in the more advanced countries (including even Third World countries like Taiwan and South Korea). India has about the same overall quality of natural environment — land, water resources, temperature, etc. — as the United States; it also has roughly the same arable acreage. Twenty per cent of its land is under irrigation (in comparison to 10% in the US), and this could easily be doubled in the 1980s — at a time when worldwide additions to irrigated acreage are expected to slow sharply as compared to the past 25 years.

"India, therefore, could be producing at least three times more grain per acre than it is now doing, at present cost levels, if it

could overcome certain problems of organisation, finance, and applied research. This would increase its total output from the current level of 120 million tons of grain to more than 300 million tons (compared to a present US level of 250-260 million tons)."

Indian should actually attempt to do so, argues Grant, because there is the growing need for an alternate breadbasket for the world. The International Food Policy Research Institute, Washington, has estimated that the Third World's food imports will triple to about 120-145 million tonnes every year by 1990, and this may be beyond North America's capacity to supply alone.

The success that has been achieved already is to a great extent the result of investments made in agricultural research. The ICAR was set up in 1929 by the British colonial government — but nobody even knew in 1964 "where the ICAR was in the organisation chart of the government", to quote a senior Indian science planner. It was then a small 'attached office' in the Ministry of Agriculture, totally bureaucratised and emaciated. But in that year, the ICAR was reorganised as part of the country's drive towards the green revolution, and expenditure on agricultural research by ICAR rapidly grew from Rs 64 million in 1965-66 to Rs 370 million in 1976-77 (though still less than the amounts spent on either defence, atomic energy or space research). Research is now conducted through a grid of agricultural universities, 29 central research institutes and numerous all-India coordinated research projects sponsored by the ICAR.

Indian agricultural scientists have received considerable support in their work from international institutes like the International Maize and Wheat Improvement Center in Mexico and the International Rice Research Institute in the Philippines, but they themselves have conducted substantial research to adapt imported genetic material to local environmental conditions, find new high yielding varieties (HYVs), and develop the agricultural practises required to support them. International agricultural R&D centres have now multiplied to eleven, but recognising that they cannot take the role that national research organisations should play, as shown by the work of ICAR, the Consultative Group on International Agricultural Research will set up a new institution called the International Service for National Agricultural Research (ISNAR) in 1980. ISNAR will mainly help to develop national crop research centres.

But successes notwithstanding, Indian agricultural research faces even stronger challenges in the years ahead. It is important, for instance, to develop more biological sources of nitrogen fixation like blue-green algae, which reduce the need for

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Increasing rice production by for a second crop

expensive chemical inputs. Without this type of new agricultural technology, most small and marginal farmers will remain seriously constrained in using the green revolution technology package. A large part of the increase that has taken place in rice production in recent years has been contributed by the rich farmers of Punjab, which is not a traditional rice-growing state. During 1978, the government procured more rice from Punjab alone than in the whole of the rest of India. Punjab's rice yields are more than double the all-India average yield. Rich Punjab farmers were also the forerunners of the green revolution in wheat. Punjab produced more wheat in 1978-79 than was produced in all India 1951-52. Eastern India, however, which is the traditional rice-growing region, has not benefitted equally from the new genetic package in rice. This region is dominated by extremely small landholdings and prone to floods, which submerges the short HYVs.

Major research into pest control

Pest and disease control is another major area of research. The danger of pest and disease invasion is much higher in tropical regions and HYVs have not only been, in general, found to be more susceptible compared to the traditional varieties, but their introduction has resulted in some minor pests, like the brown plant hopper, emerging as major threats. Integrated pest control and surveillance practices which reduce or obviate the need for pesticides will, therefore, have to be developed.

Protecting the "ecological infrastructure", as Dr Swaminathan puts it, is yet another area for careful research and planning. Intensification of agriculture and increased pressure on forests for domestic fuel and commercial

timber, particularly in the Himalayas, is now resulting in serious floods year after year. Environmentally sound agriculture and forest management in mountainous ecosystems is, therefore, of prime importance to India. But substantial research has yet to be done in this area and, even more work has to be done to introduce what is known to the local people.

Equally important is the question of soil fertility. Burning of cowdung and lack of tradition for using human excreta as manure means that organic fertilisers are not reaching the soil. In certain heavily cultivated northern states, research is now showing that even certain micronutrients like zinc are becoming constraints to increases in yields which can only be partially offset by the increased use of nitrogenous chemical fertilisers.

Research in non-cereal food also needs considerable attention. Pulses, which provide most of the protein in the Indian diet, have suffered a setback in production as farmers have increasingly turned away from pulses to grow the more remunerative HYVs of important cereals in the wake of the green revolution. Until HYVs of pulses are also available, farmers are unlikely to give them high priority. The India-based International Crops Research Institute for the Semi Arid Tropics is now actively conducting research on certain pulses. But several other minor crops that are locally important, for instance, in the hills, continue to be under-researched.

Conservation of germplasm too is a vital area. India is a rich centre for genetic variability in several important cereals, vegetables, cotton, pulses and fruit trees. With increasing deforestation and cultivation of monocultures spreading rapidly, this genetic treasurehouse stands threatened. Organisation of germplasm banks is thus of extreme importance.

Some good progress has certainly been made in research on dryland farming, largely aimed at reducing risks in cultivation in rainfed areas. Even with full utilisation of available water in the Indian subcontinent, there will be large areas that will not be able to receive assured irrigation. Even small fluctuations in the monsoon often spell disaster for farmers in rainfed areas and agriculturists in such areas are usually very poor. The ICAR has been doing extensive research in this field. It hopes eventually to provide farmers there with the option of switching over quickly to an alternate crop that can be grown in the remaining period of the crop season. This would ensure the farmer some return at least in that year.

Crop life-saving research, which aims at saving the plant in periods of acute stress, has also been showing good results. The integral part of such research is water management at the level of every watershed. Each watershed is advised to make a pond to collect the run-off and use this water to save the crop. Essentially, this is not a new advice. Most villages in ancient India have a village pond. Together with this engineering approach, efforts are also being made to develop new drought-resistant HYVs.

Vicious effects

The future of agricultural research in India is thus both bright and challenging. But whether all Indians will benefit from it equally is something that will depend much on India's social and political will. The green revolution technology has had a vicious effect on the rural poor. But to what extent the new agricultural technology is responsible for it, is not yet clear. In a highly stratified socioeconomic environment, it is more likely that any new technology which offers major productive opportunities, will be used by the more powerful to become still more powerful.

This is true, in fact, of even the age-old technology of irrigation. "Few people seem to realise that, normally, in the case of any irrigation scheme, out of every rupee worth of direct benefits accruing from irrigation, 54 paise would go to the large and big farmers who account for only 10 per cent of the total rural households", says a report recently prepared by a senior official in the Ministry of Agriculture.

Agricultural science, however, has clearly shown that it has the potential to feed India's population, and given political will and mobilisation of the people, even provide employment on a massive scale. But will the agricultural scientists get full support in their venture? It is interesting to note that after the Pokhran nuclear explosion in 1974, the government of India had quickly announced that nearly half a dozen nuclear scientists would get the Padma Shri, India's national award. But few scientists were so honoured for their work on HYVs of wheat, which changed India's agricultural future. □

Joseph Hanlon

correspondence

Berufsverbot: physics professor sacked

SIR, Professor Jens Scheer, one of the leading critics of Germany's nuclear energy program, is to be forced to leave his life-long tenured position as professor of nuclear physics at the University of Bremen. The Administrative Court of the Free Hanse City of Bremen imposed this sentence of Berufsverbot (profession forbidden) in June after a disciplinary trial and retrial lasting two years. In its ruling the three-judge panel concluded: "As an appropriate disciplinary action for this offence only the heaviest one can be considered: expulsion from office".

The "offence" appears in the Federal Constitutional Law for Civil Servants. It is only because the German civil service includes, *inter alia*, permanent university staff that professor Scheer is bound by this law. This circumstance has important and unavoidable consequences especially for those who must teach and carry out objective research. The court stated:

"It is expected of a civil servant that he recognises and acknowledges the State and its constitution as a high positive value worthwhile to defend actively; Federal Constitutional Law (Beschluss von 22,5, NJW 1975, 1641)". (This is applied to one's private life as well.) "According to this law (Art. 33 Abs. 466) a civil servant stands in public-legal relations of service and loyalty. That is why it is his duty to be loyal toward the State and the constitution (political loyalty-duty of the civil servant)."

The ideal of this law has been recurrently expressed in the last century of German History as a means to bring academics under the political scrutiny of the State authority. The present authorities have not changed history.

The nature of the trials of Professor Scheer is typical of the political trials going on in Germany today on a large scale. This latest Berufsverbot trial against him was based solely political principles and membership in the KPD (Communist Party of Germany) which is a legal party. The court noted that he "prominently", "publicly" and "actively" agreed with the principles of the party (i.e. he had recently stood as a local and parliamentary candidate and had sold the party newspaper). These were the only accusations used for the conviction and sentence. He was not allowed a complete defence.

Banning may come to those not quite bound by the golden chain of civil servant status, without court proceedings, after an investigation by the secret Federal Constitution Protection Police (BVSP) or because of accusations collected from paid informants or from fellow-teachers and pupils.

Over the past five years the authorities have brought at least seven prosecutions against Professor Scheer, one after the other. Since all these attempts failed, the authorities came eventually to rely on exclusively political accusations.

The present trials is in fact one of a series of Berufsverbot trials, where new charges have constantly been laid. The trials began before Professor Scheer became politically 'prominent'.

People who support Professor Scheer or who are terrified by the existence once again

of these laws are asking the Senate of Bremen to overturn the court's ruling. These people include the Rector of the University, at least three-quarters of the professors, a similar majority of the University Staff Assembly and many students.

There is no doubt "that the West German authorities disregard individual rights and use improper methods in order to silence their critics". There is no doubt that the Civil Servants Law and its reflection in Bremen are tools to that end. And there is no doubt that neither truth nor democracy can proceed on the basis of loyalty, discipline and obedience.

Yours faithfully,

UWE LAHL
W THIEMANN

University of Bremen

Indian science

SIR, "India: the struggle for useful science" by Anil Agarwal (23 August p 625) was objective — but gave very little account of the role of scientists in general. Though India boasts a large reservoir of talented and well-trained scientists, unemployment and under-employment among scientists are considerable. Many talented scientists fail to make any mark because of their inability to go along with the establishment. And when these scientists go abroad to prove their abilities, the establishment itself honours them. At least three Indian scientists have committed suicide out of sheer frustration in not being able to cope with the powers that be. Although everybody appreciates the role of science, no one has yet given any serious thought to the exact role and ambition of scientists in the research and developmental projects of which he is considered to be a part. Research institutions are mostly modelled on a bureaucratic pattern and, as correctly pointed out in the article, unless one turns into an administrator, a professional scientist is rarely honoured. Fortunately, there is no restriction as yet to free expression by scientists: hence this letter.

Yours faithfully,

B BANERJEE
Tocklai Experimental Station, Jorhat

Humane farming

SIR, Baker and Manwell (23 August, p630) strongly infer that I oppose "humane" treatment of farm animals by advocating intensive husbandry. This is incorrect. I am not an animal behaviourist, but I have supervised the rearing of farm animals in confinement, under experimental conditions, for many years. There are, broadly speaking, two approaches to humane treatment, pragmatic and anthropomorphic. The pragmatic approach says that inhumane treatment is counter-productive, because unhappy animals do not eat well, and hence do not thrive. Solitary confinement shows this. Chickens search frantically for a companion. In one instance, two cows would not eat when separated, but started again when a window was cut in the partition. However, companionship may be *de trop*; egg production in hens is diminished by sexual harassment by male chauvinist roosters, which are therefore kept away, except in breeding flocks.

The floor space per animal allotted under

confined conditions must be adjusted for maximum productivity, which is diminished by overcrowding. Composition and intake of food must be nutritionally optimal, drinking water always available, and ambient temperature such as to be compatible with good health. Dairy cows are notoriously sensitive, not to intensive husbandry, but to minor annoyances, which may make them refuse to let their milk down.

The anthropomorphic approach that decries crowding of farm animals is of dubious validity, in view of the fact that human beings pay large sums to be jammed together at football games and in theatres. The most successful cocktail parties are tightly packed gatherings where the noise is deafening, and hotels in the United States display signs that warn against over-occupancy of meeting rooms. The chicken that has to roam in the out-of-doors probably would choose the protection of the hen-house against foxes, coyotes and *Accipiter* hawks.

Indeed, with rare exceptions, such as myself, wilderness conservationists dislike the solitude of the wilderness, for I have noted that most devotees of the Sierra Nevada seek out the few crowded areas, such as the John Muir Trail and the summit of Mount Whitney, leaving the choicer spots to darkness and to me.

Yours faithfully,

THOMAS H JUKES
Berkeley, California

Costs of toxicity testing

SIR, Dr Conning in his letter of 2 August (p 354) draws attention to the problems facing the chemical industry worldwide, of society demanding greater assurance against the risks of error or of inadequate knowledge than it did in the past. One of the principal difficulties has been to anticipate the possible effects of a chemical on man and the environment before it was introduced into commerce, especially in those cases where the effect may become apparent only after a long latent period.

In a Discussion Document published in June 1977, the Health and Safety Commission proposed to require by statutory regulation the formal prior study and reporting of the toxic properties of substances which were to be newly introduced. This was an attempt to establish a first order of priorities, since although — as Dr Conning rightly points out — even long experience of manufacture and use does not provide an infallible safeguard, at least by tackling urgently the 'new' substances we should not be adding to the existing list of 'don't knows'. HSE does not intend to produce guidelines on how to test chemicals, but merely to indicate what data would be needed as basic information for an evaluation of the properties of such substances.

It is common ground in this field that what we need are properly validated test procedures whereby chemicals may be screened effectively and at minimum cost in order to identify the hazards which they might present in manufacture and use. Such procedures must, however, be kept flexible in order not to dissipate resources in needless evaluation of effects which could be assessed from existing data either published or held in firms' archives.

Yours faithfully,

E W LANGLEY
Health and Safety Executive, London

news and views

Joining immunoglobulin genes

from W. Dunnick

ONE classical idea in immunology is the joining of variable region genes to constant region genes. The carboxy terminal part (constant, or C region) of immunoglobulin polypeptides is sequence invariant, and is probably coded by one or a few genes. For example, 95% of all mouse light chains have a single 'kappa' C region sequence; the remaining 5% are divided between two 'lambda' C region sequences. These protein sequences are probably encoded by a single kappa C gene and two lambda C genes. The amino terminal part (variable, or V region) of immunoglobulins is highly diverse in sequence; duplicate V sequences are rare among the hundred or so mouse kappa proteins studied to date. Fifty to one thousand genes are thought to code for mouse kappa V regions. Dreyer and Bennett proposed that the immunoglobulin 'gene' is formed from these two different gene pools by translocation of DNA (*Proc. natn. Acad. Sci. U.S.A.* **54**, 864; 1965). Thus, one V gene (out of hundreds) is joined to one C gene to form the genetic unit which is transcribed and translated.

This idea was proved correct by restriction analysis of DNA (Hozumi & Tonegawa *Proc. natn. Acad. Sci. U.S.A.* **73**, 3628; 1976), and by cloning and sequencing experiments (Bernard, Hozumi & Tonegawa *Cell* **15**, 1133; 1978). Immunologists were surprised to learn that, in the germline, the last one-eighth of the V region was not attached to the rest of the V region (where it belonged), but was closely associated with the C gene. In

embryonic DNA, the DNA segment coding for the first 95 amino acids of the lambda V region was separated, by more than 4.5 kb, from the segment of DNA which included the coding sequence for the last 13 amino acids of the lambda V region (the J segment), the coding sequence for the lambda C region, and the 1,200 bp intervening sequence between J and C. In DNA of a cell producing a lambda light chain, the V segment coding amino acids 1-95 is joined to the J segment coding for amino acids 96-108 to form the contiguous V gene. The intervening sequence remaining between V and C is probably removed by RNA splicing (see *News and Views* **276**, 322; 1978, for more background and references). In the mouse, the kappa light chain system is more complicated than the lambda system; there are about 100 times more kappa V segments than lambda V segments. The analysis of kappa J segments by Max *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **76**, 3450; 1979) and by Sakano *et al.* (*Nature* **280**, 288; 1979) has revealed a system slightly more complicated than the single lambda J segment, but also more informative.

Both groups cloned a segment of embryonic DNA that included the kappa C gene and 4.5 kb upstream from the C gene. A large part of the DNA was sequenced, and five J segments were identified by homology to kappa protein sequences. The J segments are in a cluster, separated from each other by about 300 bp; the nearest to the C gene is about 2.5 kb away (Fig. 1). In the 5' direction, there are no additional J

segments within 1.1 kb; there are probably none between J5 and the kappa C gene. The data cannot exclude additional J segments elsewhere. From other data it seems unlikely, though not impossible, that additional kappa J segments exist.

J segments and joining

What recognition signals are important in V-J joining? Adjacent to the 3' end of sequenced V coding segments and to the 5' end of each of the five J segments is the palindromic sequence (or a slight variant of) C-A-C(A)G-T-G. This sequence is also found following a lambda V segment and before the lambda J segment (Bernard *et al. op. cit.*). A second consensus sequence is found 25-30 nucleotides from the J segments, G-G-T-T-T-T-G-T-A. The homology is so striking and well-preserved that one feels the presumptive joining enzyme would recognise these sequences. Using the cloned V and J segments, one might begin a search for the joining enzyme. This could be a formidable task, as no one knows in what cells V-J joining takes place, or how many of these cells can be isolated.

Of the five J segments, examples of four can be found in known kappa protein sequences. The J3 sequence is not found in any known protein. It has the most divergent sequence of the set, and the intervening sequence following J3 does not begin with G-T, even though almost all other intervening sequences do (Breathnach *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4853; 1978). Thus, Max *et al.*



Fig. 1 Arrangement of kappa light chain genes in germline and myeloma DNA. Adapted from Seidman *et al.* (*Nature* **280**, 370; 1979). This figure is not drawn to scale. Darkened boxes are coding sequences. The exact arrangement of the V segments is not known, relative to other kappa V segments or to the kappa J segment and C gene.

suggest that J3 is not expressed in protein because its intervening sequence cannot be properly excised. The correlation between expression and junction sequence is interesting, but there is a second exception to the G-T rule which is certainly excised properly and expressed (Sakano *et al.* *Nature* 277, 627; 1979). How rigid is the G-T rule? In the J3 segment, is divergence away from G-T at the beginning of the intervening sequence a cause for non-expression or a result of non-expression?

J segments and diversity

Both Max *et al.* and Sakano *et al.* have noted that V-J joining may generate sequence diversity. Bernard *et al.* (*op. cit.*) and Weigert *et al.* (*Nature* 276, 785; 1978) have also suggested the possibility. By adjusting the point of recombination within the three nucleotides following the V coding sequence and the three nucleotides of residue 96 in the five J segments, one can generate all known residue 96 amino acids in Balb/c and NZB kappa proteins. As an example, the nucleotide sequence at the end of the MOPC-41 V segment is (Seidman *et al.* *Nature*, 280, 370; 1979):

Pro 95| non-coding DNA

-C-C-T-C-C-C-

The first three nucleotides of J1 are -T-G-G-. Recombination joining the first C after the V coding segment to the two Gs in the J1 segment yields -C-G-G-. C-G-G codes for arginine, residue 96 in MOPC-173 (residues 97-108 of the MOPC-173 kappa chain are consistent with the sequences of J1). Other possible results of recombination are -C-C-C- (Pro, MOPC-11), -C-C-G- (Pro, MOPC-11), and -T-G-G-(Trp, MOPC-41). Another possible event joins the three Cs following the V segment to the three nucleotides coding for residue 96 in the J segment. For example, joining -C-C-C- following the MOPC-41 V segment to -T-A-C- at the beginning of J2 yields Pro-Tyr, the residues 96 and 96a of 7132, a NZB kappa chain which has an 'extra' J amino acid.

Does sequence diversity at residue 96 lead to new antigen combining sites? This question, though central to discussions of diversity, is difficult to answer. In the few immunoglobulins whose three-dimensional structure is known, amino acid 96 is near or at the antigen combining site (Padlan *et al.* *Immunochemistry* 13, 945; 1976). It is probably safe to assume that diversity generated by V-J joining, in some cases at least, can alter the antigen binding properties of immunoglobulins.

V-J joining is an exciting topic because it is the first example of a defined molecular mechanism which probably leads to V region sequence diversity. This is a step forward for immunology. Nevertheless, V region diversity must also be generated by other mechanisms. None of the sequence differences in mouse lambda V regions can be explained by V-J joining. In mouse

kappa V regions, 20-30 amino acid residues show more sequence variability than does residue 96. The light chain of myeloma-2413 (from a NZB mouse) has aspartic acid at residue 103 and glutamic acid at 107. Both residues are lysines in all five Balb/c germline J segments. Either the NZB has at least one different J segment, or somatic mutation must act even on J segments.

From work during the past three years, culminating in the papers by Sakano, Max and their colleagues, we know a great deal about light chain J segments in the mouse genome. Protein sequence data suggests that heavy chains also have J segments (Rao *et al.* *Proc. natn. Acad. Sci. U.S.A.*

76, 2890; 1979), and recombination has been postulated to occur near residues 96-104 in human kappa chains (Milstein *Nature* 216, 330; 1967). Several questions concerning J segments will be answered by studies in these areas. How many J segments do the kappa and lambda systems have in humans, where the two light chains are about equally represented in serum immunoglobulin? Does each heavy chain C gene have its own J cluster? Does the role of J segments in diversity extend beyond V-J recombinations? □

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Sun shines on photosynthetic prokaryotes

from A. J. Smith

THE clear message to emerge from recent studies with photosynthetic prokaryotes is that there is much more to the microbial world than *Escherichia coli*. Of particular interest is the application of recombinant DNA techniques to the analysis of the genetics of these organisms. At a recent symposium* B. Marrs (Saint Louis University, Missouri) described how a promiscuous plasmid with sex factor activity has been used to mobilise segments of the chromosome of *Rhodospseudomonas capsulata* including that carrying the genes for photopigment synthesis, reaction centres and tryptophan synthetase. The cloning of portions of this DNA for use in studies of gene structure, function and regulation is now possible following the isolation of R-prime plasmids in which the photopigment genes are incorporated into the plasmid structure. The genetic analysis of cyanobacteria has been handicapped by difficulties in the isolation of mutants and the lack of efficient systems for gene transfer other than transformation which has been demonstrated in a number of strains. R. Haselkorn (University of Chicago) has avoided these problems in an investigation of the genes for nitrogen fixation (*nif* genes) in an *Anabaena* sp. He identified fragments of cyanobacterial DNA containing *nif* genes by hybridisation with recombinant plasmids containing defined segments of the *nif* region of *Klebsiella* DNA and found a 10 kb fragment which contained sequences complementary to *Klebsiella* genes *D*, *H* and *V* (genes *D* and *H* code for components of nitrogenase). Sequences complementary to genes *Q*, *B*, *A*, *F*, *M* or *S* were not found in any of the DNA fragments, suggesting that the *Anabaena* counterparts of these genes have diverged too far to be studied by this technique. The discovery of closed covalent circular DNA (plasmids) in a variety of cyanobacteria (G. A. van Arkel, State University, Utrecht; F. Doolittle,

Dalhousie University, Halifax, Nova Scotia; V. Saunders, Liverpool Polytechnic), has introduced the possibility of using them to mobilise sections of the chromosome of these organisms. The Utrecht group reported the successful incorporation of an ampicillin-resistance marker of the *E. coli* plasmid pR146 into a small 53,000 molecular weight plasmid in *Anacystis nidulans* R-2. In the gene transfer system they are currently developing, this modified plasmid will be used as the cloning vehicle for fragments of chromosomal DNA by the technique of insertional inactivation. This insertion of the complete sequence of the Tn901 transposon into the cyanobacterial plasmid along with the ampicillin resistance marker may yield a new method for producing mutants if the jumping character of the DNA segment is preserved in its new location. These recent advances will open the way for analyses of genes specifying the photosynthetic apparatus, nitrogen fixation and the mechanisms for their regulation as well as the characterisation of processes governing the development and differentiation of photosynthetic prokaryotes.

Photosynthetic bacteria and cyanobacteria include organisms which are being used as model systems for the study of morphogenesis and differentiation as indicated by the review papers on *Rhodospirillum rubrum* (C. Dow, University of Warwick) and cyanobacteria (C. P. Wolk, Michigan State University). Factors determining the production and spacing of heterocysts, which are the sites of nitrogen fixation in certain filamentous cyanobacteria, have yet to be fully characterised. Furthermore, the identity of the reductant that passes from vegetative cells to the heterocyst to support nitrogen fixation and the origin of glutamic acid which is used to export combined nitrogen in the opposite direction have yet to be established with certainty. Reports of reproducible procedures for the production and germination of akinetes (M.

*The Third International Symposium on Photosynthetic Prokaryotes was held on 27-31 August in Oxford and was organised by N. G. Carr (University of Liverpool.)

Herdman, Dundee University) which are a form of non-growing resistant cyano-bacterial cell indicate that the analysis of this further example of prokaryote differentiation is now possible.

Recent work on pigment organisation and primary photochemistry which was reviewed by P. Thornber (University of California, Los Angeles) highlighted the specialised features in some photosynthetic prokaryotes of the antenna complexes which harvest light energy and channel it into the photosynthetic reaction centres. Chlorosomes (formerly known as *Chlorobium* vesicles) which fulfill this function in green photosynthetic bacteria consist of a relatively unorganised assemblage of bacteriochlorophyll *c* molecules in a non-unit membrane-bound sack positioned in close proximity to bacteriochlorophyll *a* – protein complexes in the cytoplasmic membrane. The antenna complexes of cyanobacteria and red algae are known as phycobilisomes. D. Bryant (Pasteur Institute, Paris) proposed that these highly organised complexes of several types of phycobiliprotein consist of a triangular core of allophycocyanin molecules with three peripheral rods radiating from each of two sides of the triangular core, the rods being made up of phycocyanin and of phycoerythrin. The sharing of certain carriers between photosynthetic and respiratory electron transport which has been demonstrated in photosynthetic bacteria may prove to be a feature of cyanobacteria as well; the dark aerobic utilisation of molecular hydrogen by *Anabaena cylindrica* is likely to involve the transport of electrons along the section of the carrier chain containing plastocyanin and plastoquinone (H. Bothe, University of Cologne).

The factors that play a critical part in establishing the dominance, albeit transient in some cases, of natural populations of photosynthetic prokaryotes are ill defined. Recent field and laboratory studies have introduced the real possibility of interpreting ecological phenomena in terms of the physiology and biochemistry of photosynthetic prokaryotes. H. van Gernerden (University of Groningen) proposed that the availability of acetate is a factor likely to contribute to the appearance of blooms of *Chlorobium phaeobacteroides* in Lake Kinneret. Other reports attributed the survival of a large population of the cyanobacterium *Oscillatoria limnetica* in the 'solar lake' in Israel despite extreme and rapid fluctuations in conditions to the metabolic flexibility of this organism. Besides the ability to switch from anoxygenic to oxygenic photosynthesis (B. B. Jorgensen, Hebrew University, Jerusalem), this organism is also able to catabolise endogenous polysaccharide reserves either by an aerobic

respiratory mechanism or by a lactate yielding fermentation under anaerobic conditions (M. Shilo, Hebrew University, Jerusalem).

In the session on evolution R. E. Dickerson (California Institute of Technology, Pasadena) maintained a lively hope that protein sequence studies would, in due time, yield data of phylogenetic significance, the accumulation of new information serving to minimise the blurring effect of lateral gene transfer. This final session closed with a progress report delivered in inimitable style by R. Lewin (La Jolla, California) on the characterisation of prochlorophytes which are associated with colonies of didemnid ascidians. Their seemingly opposed affinities on the one hand to bacteria in terms of ultrastructure and on the other to green algae in terms of their photosynthetic apparatus has been further confirmed by work in various laboratories. The outstanding requirement here is for conditions to be identified which will transform these ecological eccentrics into creatures amenable to laboratory investigation. □

Photosynthesis research in the USSR

from D. O. Hall

IN the USSR a massive effort is being put into photosynthesis research in all its various aspects. To improve crop yields, physiological and agronomic aspects must be understood; if biomass is to be used as a source of energy photosynthetic efficiencies must be improved; and if photosynthesis is ever to be mimicked to provide 'futuristic' energy, for example hydrogen and fixed carbon and nitrogen, the basic processes of photosynthesis must be understood. All these aspects of research into photosynthetic mechanisms are being undertaken in the USSR in a seemingly determined way.

A travel exchange between the Royal Society and the USSR Academy of Sciences recently enabled me to visit three centres of photosynthesis research — the Moscow-Pushchino region in Russia, Tashkent in Uzbekistan, and Kiev in the Ukraine, all of whose research has some relevance to solar energy utilisation. The huge Biological Centre at Pushchino incorporates the Institute of Photosynthesis, which to my knowledge is the only such Institute in the world devoted solely to the study of photosynthesis. A new young director Y. E. Erokhin was recently appointed and has responsibility for several hundred research workers and technicians in the departmental groupings — photochemistry, biochemistry, molecular organisation and structure and physiology. The many laboratories specialise in most aspects of photosynthesis, from whole plants down to biophysical work on pig-

ments and membrane structure.

The first report of a thermostable hydrogenase (from the red photosynthetic bacterium *Thiocapsa roseopersicina*) emanated from I. N. Gogotov's laboratory at Pushchino. They showed that this hydrogenase had a temperature optimum of 75 °C and was quite stable in air — before this hydrogenases were thought to be very unstable. Now a range of hydrogenases have been reported from various organisms which are reasonably stable and are thus useful in biophotolytic systems which evolve hydrogen gas. This is an active area of research in Gogotov's laboratory as it is part of the Soviet Union's solar energy programme.

Phytotrons, computer facilities, and special equipment services go to make up a very impressive Institute. A detailed report on its activities will soon be available in English. Two other Institutes in Pushchino — those of Biochemistry and Physiology of Microorganisms, and of Protein Research usefully complement the photosynthesis research groups.

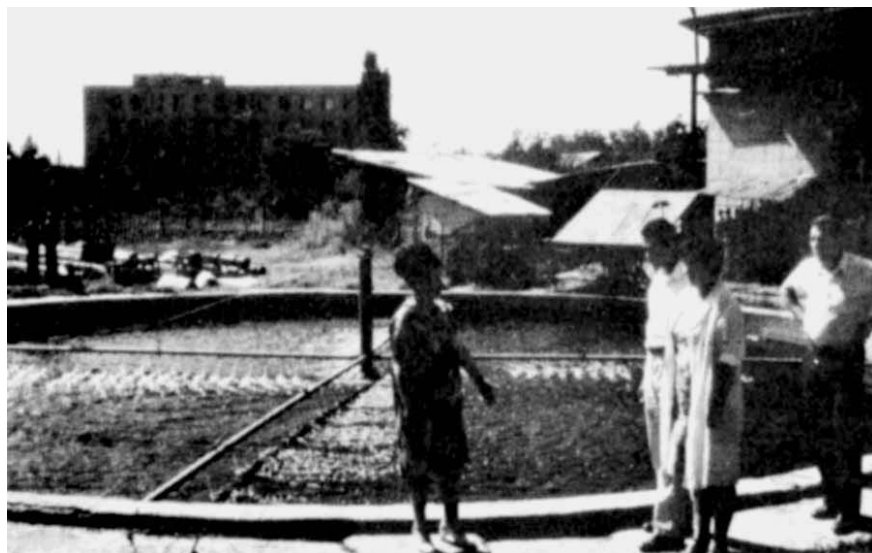
In Moscow the Academy's Institute of Biochemistry houses the Laboratory of Photobiochemistry under Academician A. A. Krasnovsky (of Krasnovsky reaction fame — the photochemical reduction of low potential compounds using artificial chlorophyll systems). Krasnovsky's laboratory is studying hydrogen-producing systems using model pigment systems, for example, chlorophylls, TiO_2 particles, viologens, ferredoxins, hydrogenases and platinum. They were the first to show in the 1960s that TiO_2 , ZnO and WO_3 could split water with ultraviolet light, evolving oxygen and reducing ferric and quinone compounds. Work continues in placing such systems in micelles and liposomes and on multilayers. At Moscow State University the Laboratory of Chemical Enzymology in Berezhin's Chemistry Department is also looking at biological energy converting systems.

Work is progressing on trying to stabilise chloroplast membranes (not yet totally successful, as everyone is finding out) and on growing algae in ponds for subsequent fermentation to methane. They also have an active group on the construction of fuel cells using hydrogenases immobilised on gels and graphite electrodes; an oxidase, for example, laccase, comprises the other half cell. Such fuel cells seem quite stable and operate at high efficiencies.

In Skulachev's Laboratory of Bioenergetics model membranes as photoconvertors are an active area of research. In the Department of Microbiology, Professor E. Kondratieva heads a famous group working on C, H and N metabolism of photosynthetic bacteria and on methane-producing bacteria. The growth of photosynthetic bacteria under dark and light conditions and under various oxygen

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Experimental algal pond in Tashkent

tensions has an important bearing on their ability to fix N_2 and evolve H_2 — besides using various carbon compounds as a substrate source. This laboratory has shown that *Thiocapsa* can be grown chemoautotrophically in the dark under oxygen but it needs thiosulphate and CO_2 — it cannot use organic compounds under such conditions. This relates to Pfennig's work in Göttingen on the non-sulphur *Rhodospseudomonas* which can grow in the dark with H_2 and CO_2 when oxygen concentrations are low. These observations seem important since they point to an adaptability of photosynthetic bacteria not hitherto realised. Kondratieva's laboratory is also actively involved in the possible utilisation of photosynthetic microorganisms in solar energy conversion systems.

In the Academy's Institute of Plant Physiology in Moscow, Professor A. A. Nickiporovich heads the Laboratory of Photosynthesis which has a long history of work on factors determining the efficiency of photosynthesis and plant productivity. Nickiporovich heads the group in the USSR Solar Energy Committee looking at biomass as a future source of energy, while, Krasnovsky leads the group investigating the potential of photosynthetic mechanisms as solar energy converters.

The Republic of Uzbekistan has over 400 algal ponds in operation today. It was an eye-opener to visit the Institute of Microbiology at Tashkent headed by Academician A. M. Muzofarov and run by the Uzbekistan Academy of Sciences in Tashkent. Besides practical research in mycology (the first volume of floral mycology of Uzbekistan having just been published) and fermentative microorganisms, there is a significant effort on algal ecology, physiology and productivity.

Ponds growing green algae under thermophilic and mesophilic conditions apparently operate in many parts of the Republic to provide food supplements for cattle, chickens and silkworms. Yields vary from 30 to 60 tonnes dry weight per hectare per annum, the limiting factor being low

winter temperatures of $2-5^\circ C$ although there is still considerable sunshine. A large system near Samarkand produces a tonne of wet cells per day while much larger ponds are under design. Interesting experiments are also being done with blue-green algae, with water plants like *Lemna* and with fertilisation of cotton with algal suspensions.

At the Ukrainian Academy's Institute of Plant Physiology at Kiev, Professor Grodzinsky heads a team of 400 people who work on various aspects of the agronomy, nutrition, physiology, biochemistry, chemistry and physics of plant growth. Two of the eleven science departments — physiology and ecology, and biochemistry — are directly concerned with photosynthesis. Optimum conditions for productive growth under ideal and stress conditions have been analysed to help in plant selection and agricultural programmes of the Ukraine — which is the largest producer of sugar beet in the world. The chloroplast membranes and their photosystem particles and lipids are being actively studied to try to understand the inherent stability of such membranes in light and also how they might be used in artificial water splitting systems for solar energy conversion.

In Professor Ostrovskaya's laboratory there is an active group studying the localisation in and isolation of photosystems I and II from the chloroplast membranes; it seems that for photosystem II, proteins are more important for stability than the lipid environment. A photosystem II fraction has been isolated which has no contaminating photosystem I particles — this is important for spectroscopic studies.

In Professor Gulaev's laboratory they are looking at the factors limiting photosynthesis in the leaf. Seven factors can be listed and their theoretical and practical interactions are being studied. Such practical work is important in trying to select plants for optimum field growth and also under artificial conditions such as greenhouses where CO_2 fertilisation and

waste heat, from power stations for example, can be used to maximum effect.

Many other centres of photosynthesis research exist in this vast country. Three of which I am aware are the Botanical Institutes in Leningrad in Russia, Baku in Azerbaijan, and Minsk in Byelorussia and there must be many more smaller photosynthesis laboratories. My impression is that photosynthesis research has been recognised as being important to the future agricultural, ecological and solar energy prospects of the USSR. □

Galactic X-ray sources

from A. N. Parmar and P. A. Lamb

ASTRONOMERS are becoming increasingly aware that to understand X-ray sources better they must observe them over the entire electromagnetic spectrum. Participants at a recent multidisciplinary meeting* presented the results of observational and theoretical studies ranging from the radio to the gamma-ray region of the spectrum.

The evolutionary model for a massive binary X-ray source begins as a $20 M_\odot$ and a $8 M_\odot$ star. The more massive star evolves to fill its Roche lobe and transfers its hydrogen atmosphere to the secondary, which becomes the more massive component. At this stage the system consists of a massive hydrogen and a helium star, which rapidly collapses and probably explodes as a supernova, leaving behind a neutron star. The hydrogen star becomes a supergiant with a strong stellar wind, providing material which accretes onto the neutron star: this powers an X-ray source for about 5 million years.

There are many problems with such a simple model. Mass conservation is assumed, although it is known that many such stars are over-luminous for their masses, which implies that they lose mass at a high rate. The effect of mass loss on stellar evolution is not at all clear but some preliminary results were given by E. P. J. van den Heuvel (Amsterdam). The model does not explain the origin of the slow pulsars (with periods of order of a minute) although some mechanisms to slow down newborn, rapidly spinning neutron stars (which have periods of fractions of a second) have been proposed.

Several contributors demonstrated how observations over a particular wavelength range can contribute to our understanding of X-ray sources. The search for radio emission from X-ray sources was covered by B. Burk (Massachusetts Institute of Technology). In a typical binary system undergoing mass exchange X rays are

An Advanced Study Institute on Galactic X-ray sources, was held at Cape Zion on 28 May-9 June, and sponsored by NATO, The Greek National Committee for Astronomy and University College London's Department of Physics and Astronomy.

often produced in the high density, high temperature, accreting plasma close to a compact star while the radio emission is formed further out by the interaction of the relativistic plasma and the magnetic fields that are generally present. The well known X-ray binaries, Cyg X-1, Cyg X-3, Cir X-1 and Sco X-1 as well as GX17+2, the Galactic Centre and various RSCVn stars have been detected at radio frequencies.

The optical characteristics of high mass binary systems were discussed by J. B. Hutchings of the Dominion Astrophysics Observatory (DAO). Detailed spectroscopic studies of eight of the hot supergiant components indicate that although these primary stars are undermassive (compared with normal stars of the same spectral type) their elemental constituents are apparently normal. The transfer of mass is made evident by the emission lines at $\lambda 4684$ (He II) and the $\lambda 4640 - 4650$ (C III — N III) blend. Both emissions almost certainly occur in the accreting matter.

Analysis of optical light curves for these systems is complicated by the need to correct for non-circular orbits, tidal deformation, X-ray heating, non-synchronous rotation of the primary and the presence of an accretion disk.

A. Cowley (University of Michigan) presented a review of optical investigation of low mass binary X-ray systems. These fall broadly into three groups: first, those in which the optical output from the disk dominates that from the 'normal' star. The optical spectrum then consists of a hot, blue, relatively featureless continuum with $\lambda 4686$ He II emission. An example of such a system is Sco X-1 for which spectroscopic radial velocity measurements imply an orbital period of 0.782 days. However, no eclipse (or pulse) periodicity has been detected in the X-ray data. The second type of system is where the optical emission is dominated by the 'normal' star, for example Her X-1 and Cyg X-2. The latter system contains a low mass population IIF star which fills its Roche lobe. A 9.8 day orbital period has been determined for this system by radial velocity measurements. The third type of low mass systems are the cataclysmic variables. These consist of the AM Her types (with strong emission lines and magnetic fields), SS Cyg type systems (common dwarf novae) and some of the X-ray transients (for example AO620-00).

A. K. Dupree of the Smithsonian Astrophysical Observatory (SAO) reviewed recent IUE and Copernicus observations of X-ray binaries. Such observations can lead to a better understanding of many problems such as the interaction between X rays and a radiatively driven stellar wind. High temperature material in an accretion disk or X-ray heated photosphere can also be observed in the ultraviolet. Changes in the ultraviolet spectrum of Vela X-1 may be caused by X-ray photoionisation and heating of the stellar wind. The effect of X rays on the interstellar medium has been detected in observations of both

this source and 4U1700-37. Two narrow, highly excited, C IV and Si IV interstellar lines have been detected with absorption features separated by 50 km s^{-1} . These may be produced by the expansion of a 'bubble' of ionisation which expands as the X-ray source photoionises the interstellar medium. Ultraviolet analysis of the chromospheric emission from many of the hot single stars, which are now being found to be X-ray emitters, is an important new means for investigating the intriguing problem of the formation of the coronae.

Observations using the HEAO-1 and Einstein satellites have identified new classes of X-ray emitting objects and made possible a more comprehensive survey of known types of source. The emission of X rays from RSCVn, flare and main sequence stars has now been verified. About 80 discrete sources can be seen in the nearby galaxy M31, whereas the last generation of X-ray instruments are only able to detect the whole galaxy as a single source.

J. Grindlay (SAO) reported recent results on the enigmatic X-ray sources seen in some globular clusters. With the increased positional resolution of the Einstein Observatory it has been possible to measure the offset of the X-ray source from the centre of the optical cluster with precision. From an assumption that the stars in the core have an isothermal velocity dispersion, he has calculated the mean mass of this class of X-ray source. This turns out to be of order $20 M_{\odot}$. There is, however, a large error associated with this

value ($2 M_{\odot}$ is allowed for with a confidence of 10%) owing to the uncertainty in position of the optical centre of each cluster.

Previous Copernicus results which have tentatively identified OAO1653-40 with the massive binary V861 Sco were supported by more recent data from the same satellite which was presented by A. Parmar (MSSL). This showed an ultraviolet burst, which in one C III line alone had a luminosity of $2 \times 10^{37} \text{ ergs s}^{-1}$, occurring simultaneously with an X-ray turn on. If it can be shown that V861 Sco is indeed associated with the X-ray source then it becomes a firm candidate for a black hole of mass between 7 and $13 M_{\odot}$.

H. Bradt (MIT) described the detection of rapid aperiodic variability in Galactic X-ray sources. This has long been known to characterise Cyg X-1 and Cir X-1 although, with the recent detection of pronounced flaring and millisecond bursts, it also seems that the time variability of these sources is significantly more complicated than was once realised. In the past year, rapid aperiodic variability has been observed from GX301-2 and GX339-4. A 7 to 8 second delay between the arrival times of the optical and X-ray signals from Sco X-1 has been interpreted as the light travel time from the compact body to the primary. Correlated optical and X-ray activity has been found by S. Ilovaisky (Observatoire de Paris, Meudon), when the source flares.

Seven of the 16 known X-ray pulsars have had their orbits accurately measured by Doppler analysis of their varying pulse periods. One source, which may be a system containing a neutron star and a low mass white dwarf is 4U1626-67. S. Rappaport (MIT) reported on the search for orbital motion and the detection of as yet unexplained quasi-periodic bursts with a 1000 s characteristic timescale, which could be caused by material in unstable orbits which decay onto the neutron star.

Rappaport has combined data from the Ariel 5 and SAS-C satellites, and obtained a revised orbit for GX301-2 (4U1223-62) which has been determined with a most probable period of 40.8 days and a non-zero eccentricity. Optical photometry on the other hand suggests a period of about 20 days. Vela X-1 has been shown to have an eccentric orbit. Future observations of the change in the longitude of periastron will enable the amount of central condensation of the primary star to be calculated.

Detailed analysis of the X rays detected during an optical flare from the dwarf nova SS Cyg undertaken by F. Cordova (Caltech) shows the 9 s pulses to have a very sinusoidal shape. However, the pulse phase is unstable which makes a determination of the orbit very difficult. Similar



A hundred years ago

ONE feature of the last eruption of the remarkable volcano of Kilauea, in the Sandwich Islands, is the fact that the great molten lake of lava, occupying a huge caldron nearly a mile in width, and known as the "South Lake," was drawn off subterraneously, giving no warning of its movements and leaving no visible indication of its pathway or the place of its final deposit. "Other eruptions," writes Dr. Coan to Prof. Dana, in a letter dated June 20, "have blazed their way on the surface to the sea, or while on their subterranean way have rent the superincumbent beds, throwing out jets of steam or of sulphurous gases, with here and there small patches or broad areas of lava. But as yet no surface-marks of this kind reveal the silent, solemn course of this burning river. One theory is that it flowed deep in subterranean fissures, and finally disemboved far out at sea.

From *Nature* 20, 25 September,
513; 1879.

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Problems in hominid taxonomy

WE are pleased to have had our recent systematic assessment of hominid evolution recognised in *Nature* (News & Views, 278, 400; 1979). Your Paleoanthropology Correspondent has, however, provided the readers of *Nature* with a misleading analysis of our published work as well as a misunderstanding of systematic procedures followed therein.

Your correspondent was not familiar with the authorship of the formal designation of the new taxon, *Australopithecus afarensis*, Johanson, White and Coppens, 1978 (not Johanson and White as reported). The correspondent also seems unaware of the difference between diagnosis and description in a systematic publication. Features of the dentition (large mandibular canines, three-rooted upper premolars and molar size gradients) and postcranium (lack of third metacarpal styloid process, os coxae anatomy) taken by your correspondent as diagnostic of *A. afarensis* are detailed in the description of the species and not the diagnosis. Of the suite of characters distinguishing *A. afarensis*, the correspondent mentions only a few dental features and completely neglects diagnostic cranial and mandibular anatomy.

Unfortunately, treatment of our *Science* article (203, 321; 1979) fares no better as several errors, in fact, mar the report. For instance, the correspondent reports that the Taung specimen does not have a P_4 but that we measured it. In fact, the specimen has two P_4 s, but we did not measure them because they are unerupted. Similarly, our table of dental metrics (page 322) shows central incisor measurements for five individuals instead of the two reported by your correspondent. The correspondent also states that the *A. afarensis* P_3 "has a large outer cusp and a very small inner cusp". The relevant citation from *Science* (page 322) is "A smaller lingual cusp is usually present, but some specimens (A.L. 288-1, 128-23) display only an inflated lingual ridge." The fact that this feature reminds the correspondent of Miocene hominoid P_3 s emphasises our assertion that the CP_3 complex of *A. afarensis* is an important character suite that allies the new taxon with extant and extinct apes and distinguishes it from members of other hominid species.

Finally, the correspondent suggests that variation (not variability) in the Hadar sample is excessive and indicative of taxic diversity. We direct the reader to our *Science* paper

where all of our metric and morphological comparisons of the Hadar and Laetoli samples with those of extant hominoids support the interpretation that the fossil hominid material is best considered a single, naturally variable, sexually dimorphic taxon.

Nature's policy during the controversy over the status of the Taung skull was "No notice is taken of anonymous communications". (*Nature* 116, 462; 1925) We favour that forgotten policy.

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OUR CORRESPONDENT REPLIES: While I thank Johanson and White for their response to my recent News and Views comment I must point out that their reply does not effectively refute any of the major points of that comment. One of the major premises of that comment was that the diagnosis of the new species (Johanson, White & Coppens *Kirtlandia*, 28, 1; 1978) does not adequately differentiate it from *A. africanus*, the closest comparative taxon. If that premise can be substantiated then the validity of *A. afarensis*, as a separate and distinct taxon, can be questioned. The necessity of a clearly stated differential diagnosis was noted by the authors when they quoted Mayr's imperative to "list the most important characters or character combinations that are peculiar to the given taxon and by which it can be differentiated from other similar or closely related ones" (*op. cit.*, page 8). Diagnoses in the general palaeontological literature show that most workers follow Mayr's dictum. Yet precise differential statements are missing from this diagnosis; those features which qualify *A. afarensis* as a separate species have not been made clear.

Moreover, characters which may have a high degree of taxonomic significance have been described differently in the original diagnosis and in a later publication. The size of the inner cusp of the *A. afarensis* P_3 is a case in point. In the diagnosis of the new species it was stated that this feature "is often expressed only as (an) inflated lingual ridge (*op. cit.*,

page 6). In a later publication the "often expressed" ridge had become a "usually present" cusp (Johanson & White *Science* 203, 322; 1979). They cannot have it both ways and the fact that they have described the same important character state differently in two publications does not add strength to their taxonomic claims.

Further confusion in their work is apparent when, in their response, they claim that five individuals were used in obtaining measurements of the upper first incisor. Reference to my comment will show that part of that discussion centred on their claim that *A. afarensis* had broad upper first incisors. The breadth of this tooth is measured in the mesiodistal orientation and reference to their Table 1 (*Science* 203, 322; 1979) will show that four teeth were measured in this orientation, not five individuals (presumably yielding 10 teeth as they now inexplicably claim).

Moreover, Johanson and White suggest that I have confused, in my comment, the diagnosis of the new species with its description. Again, it is they who are confused. That stated that "certain traits... (were) placed in the description due to the lack of comparative anatomical specimens from other species of *Australopithecus*". (*Kirtlandia* 28 8; 1979). In my comment I pointed out that in characters of the hand, wrist and os coxae not only do comparative specimens exist for *A. africanus* but they are virtually identical in the two species.

The second major point of the News and Views comment did not concern the validity of the new taxon but centred instead on the number of taxa which may be contained in it. The authors acknowledge that the metric and morphological ranges may be broad in *A. afarensis* but they claim in the response that "all" of their comparisons between the ranges in the fossil sample and in living hominoids have supported attribution of the fossil material to a single taxon. They do indeed present (in footnotes) coefficients of variation for three features demonstrating that, in selected features, the range of variation in *A. afarensis* does not exceed that of living hominoids. Figure 6 which purportedly "clearly" (*Science* 203, 329, footnote 23) compares the range of variation for one *A. afarensis* feature with that of other hominoids is, in fact, an illustration of two distal femora.

In the end, however, the *A. afarensis* sample is an extremely important one and disputes over its taxonomic placement do not minimise that importance. Johanson and White are to be praised for their rapid publication of this material.

phenomena, described by T. Chester (Caltech) have been observed from another dwarf nova U Gem. He presented a technique for modelling these variations which preserves the phase of harmonics. Improved upper limits for higher harmonics of the pulses can be extracted.

The gas flow from a primary star to a compact body is complicated and poorly understood, making the interpretation of X-ray spectra very difficult. Spectral features from atomic transitions in iron and other elements have been observed in galactic X-ray sources. With the advent of more sensitive spectrometers, such as on the Einstein satellite, many more spectral

features are being discovered. R. McCray (Colorado) showed how the analysis of spectral features will allow a determination of the physical conditions in, for example, the flow of accreting material near a compact object. The 'standard model' (appropriate to the solar corona) of an optically thin plasma, in collisional equilibrium, does not hold near to a compact body or in a supernova remnant. An alternative model, which is similar to that for a planetary nebula, is a central point X-ray source which photoionises its surroundings into highly excited states, even at low temperatures. Here, the Auger effect and fluorescence will be very

important and radiative recombination lines may dominate the spectrum.

N. Shakura (Sternberg Astronomical Institute) has calculated the characteristic scale of turbulence in a disk which leads to an estimate of α (the ratio of the stress to the pressure in the outer part of a disk). His result, $\alpha \sim 10^{-3}$, differs significantly from previous estimates. Simulations using the precessing, twisted accretion disk model proposed to explain the 35 day cycle of Her X-1 which were presented by J. Petterson (University of Illinois) require α to be of order unity. This is not necessarily inconsistent with Shakura's work as α may vary throughout the disk. □

review article

Contact inhibition and malignancy

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The role of contact inhibition in influencing the behaviour of malignant cells is discussed in a review. Although tissue culture cannot simulate the immense complexity of the conditions in vivo, some of the distinctive features of malignant invasion can be conveniently observed with this technique. The evidence derived from this technique indicates that defective contact inhibition of movement of malignant cells does contribute to their invasiveness.

COMPONENTS of the locomotor machinery of cells have been intensively studied, and a coherent hypothesis about how cells move is being developed. In contrast, little work has been done to characterise the locomotor behaviour of cells, principally the conditions that determine their direction of movement. This is in part because direct observation of the cells is only possible in the artificial conditions of cell culture. In particular, little attention has been paid to the aberrations of behaviour of malignant cells which can be observed in culture in terms of invasion, and which can be confidently predicted. In the context of the incoherent picture that unanalysed cell movement presents at first glance, the movement of malignant cells may not seem in any way distinctive. It may also be that the interpretation I have given to the invasive aspects of malignant behaviour *in vitro*, in terms of defective (not necessarily absent) contact inhibition, has given rise to doubts because it has seemed over-simplified. Nevertheless in my opinion the cardinal points remain, that invasion—the distinctive feature of malignant behaviour—can be observed in cell culture where normal cells do not invade and that a contribution to malignant invasion seems to be made by a defect in normal contact inhibition. These are the points I wish to discuss: first, how the extent of invasion may be assessed with various tumour types in culture and second, how their invasiveness compares with defects in the contact inhibition reactions of the various tumour cells.

Patterns of invasion in culture

The method we have used for assessing invasion involves confronted cultures consisting of two foci of cells (explants) placed about 1 mm apart. Various measurements and observations can be made after the outgrowths have met. In our technique explants of normal and malignant sarcoma fibroblasts have been confronted with a standardised chick heart fibroblast explant, which has proved best for several reasons²; the choice in any

case must be arbitrary. Six tumours have been studied in detail—mouse sarcomas 311, BAS56, S180, FS9, MCIM and a mouse melanoma. The following observations have been made on cultures fixed 24 h after fusion.

Normal mouse muscle fibroblasts confronted with the standard chick heart fibroblasts show a very high degree of obstruction, the invasion zone (the strip occupied by cells from both explants) having a mean width of only 20 μm as shown in Fig. 1a. By contrast, confronting each of the six tumours with the standard chick fibroblasts resulted in invasion zones between 130 and 290 μm wide, as is illustrated for BAS56 and MCIM in Fig. 1b, c. Nevertheless, there was still some obstruction of invasion in three of these tumours (311, BAS56 and mouse melanoma), as indicated by consideration of the between-to-side ratios (the ratio, for each explant, between the distance migrated towards the other explant and the mean distance migrated from its free sides). In these three cases, the ratios were less than unity for both the fibroblasts and tumour cells. The cells of S180, the tumour on which our original measurements were made³ (but which in our experience is indistinguishable from S37 and has perhaps become confused with it) were peculiar in this respect. The between-to-side ratio was unity for both fibroblasts and S180 sarcoma cells, indicating that invasion was completely unobstructed. Nevertheless, film analysis reveals some slight obstruction of S180 cells at the fibroblast front⁴ as will be described later.

The assessment of obstruction in the remaining two tumours, FS9 and MCIM, is complicated by their non-reciprocal behaviour when confronted with the standard chick fibroblasts. This is best illustrated using the invasion index², a measure of the distance of infiltration standardised for the outward velocity from the explant which can, of course, be used separately for both cell types and which takes a value of zero when a cell type is totally stopped by the other and 1.0 when it is totally unimpeded by the other. For 311, BAS56, mouse melanoma and S180 the invasion index was approximately the same for the tumour cells and fibroblasts in each experiment (reciprocal invasiveness) but varied from 0.2 to 1.0 according to the tumour. For FS9 and MCIM, however, the extent of invasion of the fibroblasts was markedly less than that of the sarcoma cells (non-reciprocal

This article contains editorial changes which were made by his colleague after the death of Michael Abercrombie on 28 May 1979. Reprint requests and correspondence should be addressed to Dr G. A. Dunn, Strangeways Research Lab., Worts' Causeway, Cambridge, UK.

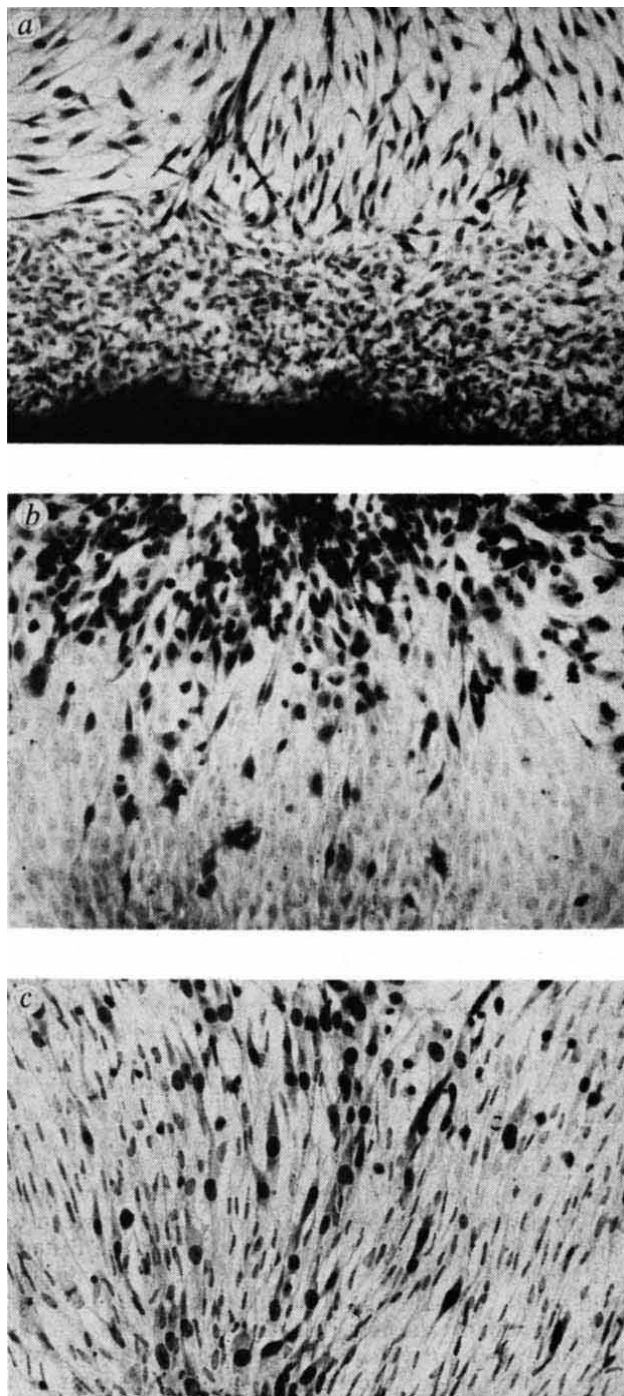


Fig. 1 Confronted explants fixed 24 h after fusion showing the invasion zone. In each case chick heart fibroblasts have migrated from below towards: *a*, Mouse muscle fibroblasts (darker nuclei)—an example of very low invasiveness, $\times 80$. *b*, Mouse BAS56 sarcoma cells (large dark nuclei)—an example of reciprocal invasiveness, $\times 80$. *c*, Mouse MCIM sarcoma cells (large dark nuclei)—an example of non-reciprocal invasiveness which also shows the effect of contact guidance (see text), $\times 122$.

invasiveness). The fibroblasts either get out of the way or are overrun. In FS9 the sarcoma invasion index was 0.6 and the fibroblast 0.1, and in MCIM the sarcoma index was 1.5 (the sarcoma cell velocity actually increased after contact with the fibroblasts because of contact guidance) and the fibroblast index was 0.2. In these two cases, the actual distance invaded by the fibroblasts was one-third to one-quarter of that of the sarcoma cells, in spite of the fact that the outward velocities of the fibroblasts exceeded those of the sarcoma cells.

Contact inhibition

The term contact inhibition refers to the phenomenon of a cell ceasing to continue moving in the same direction after contact with another cell. It is not always operative. To assess the part played by contact inhibition in invasion in culture we have to determine the obstruction to movement of the invading cells produced by contact with the normal fibroblasts. A useful 'static' measure of contact inhibition is the overlap index, which expresses the observed number of overlaps of cell nuclei as a proportion of the number to be expected if the nuclei were distributed at random. A homotypic overlap index is concerned with one kind of cell only, but of more immediate interest is the heterotypic overlap index, which is provided by the invasion zone of confronted populations. In this case obstruction is indicated when the number of nuclear overlaps of cells of the two different types is diminished compared with the random expectation of these overlaps^{3,5}. It is necessary to score nuclear rather than cytoplasmic overlaps because of the faintness and irregular outline of the cytoplasm. Since it is a requirement that the overlaps result from cell locomotion, inaccuracies in quantification can arise from the clumping of cells due to the drawing together of contractile processes (to be distinguished from the cyclic phenomenon of true locomotion) or from the occurrence of mitosis in dense cultures where the cells cannot move away. Furthermore, the presence of even small amounts of collagen between cells can reduce the effectiveness of their mutual contact and give rise to anomalous results.

Any deviation from a random distribution of freely moving cells towards a more evenly spaced distribution (that is, any overlap index of less than 100%) means that obstruction has occurred. But a single heterotypic overlap index is obtained from the number of overlaps between two cell types and, since non-reciprocal contact inhibition has been recognised, it has become clear that one of the two cell types may be obstructed much more than the other. Thus the index of overlapping may provide a distorted picture of the behaviour of the mixed culture.

A method of obtaining a 'dynamic' index from observations of living cells has been developed for detailed analysis. It necessitates classifying the results of collisions between the two types of cell as observed in timelapse filming. Although this overcomes the limitations of the heterotypic overlap index, only a limited sample of collisions can be observed in reasonable time, particularly since clearcut collisions have to be selected. Contact inhibition is observed as one or both colliding cells stopping and perhaps immediately turning aside. Stopping will also occur at random, without collision, and therefore the excess has to be estimated. At what point does the stopping occur after contact? As seen in plan view it does not usually occur immediately after cytoplasmic contact has been made. Indeed, homotypic encounters in fibroblasts commonly show a considerable degree of cytoplasmic overlap and there may even on occasion be nuclear overlapping before contact inhibition^{6,7}.

It is useful at this stage to mention a confusion that has arisen over the terms overlapping and underlapping. The overlapping so far referred to consists simply of the state of superimposition of one nucleus on another, regardless of whether the upper or lower cell, by its movement, produced the superimposition (upper and lower are themselves ambiguous terms with reference to gravity) and also regardless of which type of cell is above or below when two cell types are involved. Unfortunately the state of overlapping has been confused with the acts of overlapping and underlapping. It is perhaps best to refer to one cell as either moving outside another cell, meaning on the side exposed to the medium, or moving between another cell and the substratum. From the point of view of contact inhibition all that matters is that the cells should make contact—the form of contact is irrelevant. Bell⁸ has made a careful analysis of the origin of homotypic overlapping (crisscrossing) of Py 3T3 cells and found that the overlapping results from the movement of one cell between another cell and the substratum. Since no

estimate of contact was made, however, it is not clear how this observation leads him to call into serious question the hypothesis that tumour cells are invasive by virtue of having lost the capacity to undergo contact inhibition.

Contact inhibition in non-malignant fibroblasts

I now consider the normal background of contact inhibition amongst non-malignant fibroblasts in culture. Abercrombie and Heaysman¹ first examined the question by measuring the homotypic overlap index in cultures of two explants of chick heart fibroblasts fixed after they had reached confluence. Several other investigations using this static method were subsequently reported (see, for example, refs 9, 10). The dynamic method of time-lapse filming for examining homotypic contact inhibition was used non-quantitatively by Abercrombie and Ambrose¹¹ and then quantitatively by Veseley and Weiss¹² and by Guelstein *et al.*¹³. Heterotypic estimations with mouse muscle fibroblasts confronted with chick heart fibroblasts formed the controls for the similar work on sarcoma cell invasion already referred to². Whenever the static method has been applied to fibroblasts in a population that was not too dense, the number of overlaps observed has been very significantly lower than the statistical random expectation. This lack of overlapping is often referred to as monolayering.

It is evident, therefore, that contact inhibition is a general feature of fibroblast collision. But can it totally obstruct cell movement? Steinberg and his colleagues (see for example, ref. 14) have investigated this question in dense cultures of chick embryo fibroblasts, partly because of my own somewhat exaggerated claims which assumed that the displacements observed were largely in the form of oscillations. (There is in fact more mobility than that, many fibroblasts interchanging their positions at a speed of several micrometres per hour.) Steinberg's purpose has been, however, to establish the existence of motility in confluent monolayers—from which he concludes that obstruction is not total—rather than to make comparisons with motility in sparse populations or with migration from explants into cell-free space. Measurements by Abercrombie and Heaysman¹⁵, Gail and Boone¹⁶ and others show that when such comparisons are made, obstruction is clearly apparent.

The differential adhesion hypothesis of Steinberg and colleagues on contact inhibition amongst fibroblasts is, in my opinion, untenable as it stands. They suppose that what is inhibited is the act of passing outside and that this inhibition is due to a failure of the cell migrating outside to make effective adhesion to the dorsal surface of the cell it contacts. Typically, however, contact inhibition in fibroblasts seems to occur to a cell after it begins to pass between another cell and the substratum. Such a cell therefore undergoes contact inhibition without ever leaving its normal substratum and therefore without changing its adhesion^{16,17}. Furthermore, one of the most conspicuous features of contact inhibition in fibroblasts is the contraction undergone by the cell's front end, an event which is not accounted for by the adhesion hypothesis. A notable instance of such contraction occurs in non-reciprocal contact inhibition, where the cell that is not inhibited proceeds on its way while the cell that is inhibited (if contact is made by its leading edge) retracts, leaving the way clear in front of the first cell.

The hypothesis of differential adhesion as an explanation of contact inhibition should not, however, be discarded. It may well be important in cells other than fibroblasts^{4,12}. Even for fibroblasts, there might be an effect of a lack of adhesiveness of the dorsal surface which is masked by the contractile reaction. There are difficulties in testing whether the dorsal surfaces of fibroblasts are defective in adhesion independently of the contractile reaction. The scattering of a population of dissociated fibroblasts onto the surface of fibroblasts already adherent to the substratum fails to differentiate between the two hypotheses

since the contractile reaction may prevent the dissociated cells from spreading as they settle.

Contact inhibition in malignant fibroblasts

I now turn to consider the relation of contact inhibition to the behaviour of malignant fibroblasts.

In the experiments we have made with explants in confronted cultures, the contact inhibition between homotypic cells within any one population has an important role. The tendency of contact-inhibited cells to move towards cell-free space produced outward radial migration from an explant; non-malignant cells with high contact inhibition migrate radially with a high velocity. Most malignant cells studied also show the effect, although since their contact inhibition is commonly lower than that of non-malignant cells they do not usually show the outward drive so powerfully. Indeed, with S180 cells we have found the outward drive to be negligible. For example, a sample of cells in an S180 outgrowth showed no significant preponderance of movements away from the explant over movements towards the explant⁴. Their motility is therefore almost entirely diffusion-like in spite of their high contact inhibition, at least at relatively low density. The explanation is probably that in contrast to the other tumours investigated the majority of changes in direction of S180 cells are spontaneous rather than the result of collision between cells. This is to be expected since S180 cells are more often out of contact with each other than the other tumour cells at the same density and they show a comparatively low persistence in direction when moving freely. E. M. Stephenson (personal communication) has recently found that the outward drive is also ineffective in mouse melanoma cultures. Although homotypic contact inhibition of the melanoma cells, judging by nuclear overlaps, is also high there is only a small but statistically significant excess of movements away from rather than towards the focus.

Although much has been made of homotypic overlapping amongst transformed cells as an index of their malignancy, the correlation, if any, is by no means clear. Most observations have been qualitative. Projan and Tanneberger¹⁰ however, made an extensive quantitative study of tumours by means of the overlap index and found a broad range of indices from well within the normal range, to an extremely high level (2%–91%). This was in contrast to the indices of non-malignant cells which they found were concentrated within the range 3%–19%. It seems evident, therefore, that malignant cells are characterised by their broad variability as compared with normal cells, although their median level is no doubt distinctly higher. It should be added that it is not clear how a lowered homotypic contact inhibition of malignant cells could contribute to their invasiveness.

The high degree of obstruction between two confronted normal fibroblast explants, such as between chick and mouse fibroblasts, apparently results from their high heterotypic contact inhibition². In a free outwandering the fibroblasts move at approximately 300 μm per day, while in confronted culture they are brought almost to a standstill. How far then, can the invasion of sarcoma cells in confronted culture be accounted for by defects in their heterotypic contact inhibition?

In sarcomas 311 and BAS6 and the melanoma, the between-to-side ratios indicate some obstruction of invasion and there is a relatively low heterotypic overlap index indicating the presence of contact inhibition² (melanoma data from E. M. Stephenson, personal communication), although both the extents of invasion, and the overlap indices, are higher than with two confronted normal fibroblast explants. There is no indication of any non-reciprocal inhibition, and the invasion is approximately mutual. Furthermore there is a characteristic diversion of cells at the junction of the two populations, both cell types turning in an orthogonal direction, which is probably a manifestation of their tendency to move towards cell-free space. It seems likely that because of the incidence of heterotypic contact inhibition, the outward drive from the sarcoma explant is largely arrested by

the fibroblast obstruction, invasion being by a diffusion-like movement.

In S180 the between-to-side ratios gave no indication whatever of obstruction and, correspondingly, there was no deviation in heterotypic overlap from random expectation¹. Later studies by film^{4,11} showed a complete absence of contraction and paralysis of the ruffled membrane on contact on the part of either sarcoma cell or fibroblast. The films showed, however, a slight slowing down of the sarcoma cells while they were amongst the fibroblasts and a slight obstruction where they met the free edge of the fibroblast population. An unusual aspect of the invasion of S180 is that the sarcoma cells are almost invariably outside (on the dorsal surface of) the fibroblasts, which may well be a consequence of the fact that the sarcoma cells are rounded and project very much further above the substratum than do the flattened fibroblasts. There is no mutual diversion of the two populations.

In contrast to the three tumours (311, BAS56 and the mouse melanoma) which showed reciprocal invasion, two others, FS9 and MCIM, showed non-reciprocal invasion. Significantly, these two tumours showed non-reciprocal contact inhibition. In FS9, the invasion indices revealed that the fibroblasts were almost totally obstructed whereas the tumour cells were only partially obstructed and, when similar cultures were studied by direct filming (Abercrombie and Turner, unpublished), the fibroblasts seemed totally inhibited on contact with the sarcoma cells though the sarcoma cells were not completely inhibited by the fibroblasts. The fibroblasts show immediate retraction on contact with the sarcoma cells when in head-on collision, while the sarcoma cells continue their locomotion. When a sarcoma cell collides with the side of a fibroblast, the fibroblast generally shows a high degree of contact inhibition; consequently, almost all the invaders are sarcoma cells. For MCIM, the other example of non-reciprocity, the invasion indices show that the sarcoma cells are probably not obstructed at all. In direct filming¹⁸ non-reciprocity is clearly seen; the fibroblasts to some extent move out of the way of the sarcoma cells after retraction, and to some extent are overlapped by them. Hence the heterotypic overlap index is relatively low even though heterotypic contact inhibition is absent or greatly reduced in the sarcoma cells. (The static measurements of overlapping can thus fail to detect some instances of failure of contact inhibition.) For this tumour the mutual diversion of cells observed with the cells of other tumours is not detectable. For both FS9 and MCIM the outward drive from the sarcoma explant clearly persists into the fibroblast explant's zone (Fig. 1c).

Increased heterotypic overlapping in other tumours has been demonstrated in a mouse ascites carcinoma and in a mouse melanoma (by overlap index⁹) and a rat sarcoma (by filming¹²). Guelstein *et al.*¹³, however, found no increased heterotypic overlapping by filming in a mouse transformed cell line.

Conclusions

It is easy to describe invasion in culture but less easy to be certain of its relevance to invasion *in vivo*. This is all the more so because there is no quantitative measure of invasion *in vivo*; one has to depend on histological descriptions. Furthermore it could be that a number of transformed cells are erroneously assumed to be malignant, thus obscuring a possibly strong correlation

between *in vitro* and *in vivo* invasive behaviour. It seems more probable however that 'benign' and 'malignant' are relative terms, spanning a continuous gradation of invasiveness.

Neither is it easy to account for the behaviour responsible for invasion in culture. I have concentrated on some of the complexities of contact inhibition of movement and their correlation with the overall pattern of invasion. An important outcome is the concept of non-reciprocity, formulated originally by Heaysman¹⁸. Its simplest interpretation requires the presence of separate emitters and receptors. Where there is no contact inhibition in either of the contacting cells, neither would have emitters or receptors for each other; where there is reciprocal inhibition, both cells would have both. The emitters and receptors must be assumed to be constant in a standard fibroblast but would differ independently as between the various cells. A tumour like MCIM, which is able to induce contraction of a fibroblast—a sign of contact inhibition—would be regarded as a strong emitter, and a weak receptor, because it is not itself inhibited by the fibroblast. A tumour like S180, producing no contraction of contracted fibroblasts, would be a weak emitter and weak receptor, and one like FS9 a strong emitter but intermediate receptor. It would be instructive to test different kinds of tumour against each other, since different kinds of fibroblasts seem to vary very little in their emitters and receptors.

In addition to contact inhibition, variable adhesiveness might play a part in determining invasiveness. There may be two effects here, a homotypic effect in which cells of the same type adhere closely to each other and so prevent penetration, and a heterotypic effect in which the invaders experience obstruction because of their strong adhesion to the invaded cells. In both cases these effects are to be distinguished from the relatively low adhesiveness which is the basis of Steinberg's hypothesis of contact inhibition. The contribution to obstruction of these two possible effects of high adhesiveness cannot be accurately determined. The hypothesis that tumour cells have a low adhesiveness has been current since the time of Coman¹⁹. It may be said, however, that since adhesion is by definition reciprocal, the non-reciprocity found in some tumour invasions is not accounted for.

Other factors probably play a minor part in invasiveness. For instance, contact guidance by cells is incompatible with contact inhibition, but when contact inhibition is low, it is possible that the tumour cells may be guided by the underlying normal cells. Indications that this may be happening have been found in filmed S180 invasions⁴. With tumours (311 and MCIM) the possible effect of some diffusible agent has been detected, there being a slight inhibition of the outgrowth of the fibroblasts before junction with the tumour cells.

The study of invasion in culture provides an opportunity for investigating the many facets of invasiveness but many important gaps remain. There has been comparatively little work on the behaviour of normal cells, and the invasion of carcinomas has not been studied at all. It should be possible to approach the *in vivo* environment of cells by using three-dimensional hydrated collagen gels as substrates. The question of the role of mitotic pressure in invasion is not suited to investigations by tissue culture. In spite of the fact that so much work was devoted in the 1920s and 1930s to the supposed growth of cultures there was never any evidence that growth as opposed to migration played any part whatever. Cells cannot be pushed along a solid substratum to which they are adhering.

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articles

Molecular abundances in IRC + 10216

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The observed molecular column-densities in IRC + 10216 can be matched by chemical equilibrium calculations for $T \sim 1,250$ K, $P \sim 100$ dyn cm⁻², and no graphite grain formation. Condensation of silicon carbide into grains may explain the low observed abundances of SiO and SiS. A compressed gaseous shell may be formed between successive dust shells lost by the central variable carbon star during each of its cycles.

THE IR object IRC + 10216 is a barely visible, cool carbon star surrounded by an expanding, dusty molecular envelope¹⁻³. Parameters for carbon stars are poorly known but⁴ $R_* \approx 1,000 R_\odot$, $T_* \approx 2,200$ K, $L \approx 10^4 L_\odot$. There are grains close to the surface of the star at around $1.5 R_*$ from the photosphere⁵. The inner optically thick IR component⁶ has a radius of $10 R_*$ at $T \approx 600$ K, whereas the outer optically thin component has a radius of $60 R_*$ at $T \approx 375$ K. Recent work⁷ replaces this two-component model by a continuous temperature distribution, but the temperatures at the corresponding radii are about the same. Much further out, the upper limit on the HCN molecular envelope⁸ is $1,200 R_*$ and the extended CO envelope^{9,10} is seen out to at least $3,800 R_*$. Since Solomon *et al.*¹¹ observed microwave CO emission, 15 further molecules have been identified in its extended envelope, and abundance estimates are available for 14 of these (Table 1). We have performed molecular equilibrium calculations in an attempt to match the observations. We wish to determine if the relative abundances can be explained by a 'freeze-out' model: this supposes that chemical equilibrium holds close to the central star's photosphere, but that as the temperature and density fall in the circumstellar wind, molecular reaction rates drop rapidly enough for molecular abundances to be 'frozen' near their equilibrium values at some point near the star.

A suitable criterion for 'freeze-out' to apply is $\tau_{\text{chem}} \gg \tau_{\text{exp}}$ where τ_{chem} is the time scale for an e-fold change in the abundance of some molecule and τ_{exp} is an expansion time scale given by the time to cross a density scale-height in the shell. For IRC + 10216, $v \approx \text{constant}$ ¹ and we assume $\rho \propto (r/r_{\text{inner}})^{-2}$, that is, steady flow. Then $\tau_{\text{exp}} \approx r_{\text{inner}}/v \approx 10^8$ s if we assume¹² that $r \approx r_{\text{inner}} \approx r_{\text{star}} \approx 10^{14}$ cm. In the outer shell $\tau_{\text{exp}} \propto r$ and so the expansion time scale is longer. Calculation of τ_{chem} requires knowledge of the key reactions producing and destroying the molecules of interest and is beyond the scope of this article. However, we note that: (1) photochemical and ion-molecule reactions are probably unimportant in this neutral dusty shell

(Wannier and Linke¹³ report severe upper limits on molecular ion abundances); (2) if all neutral bimolecular reactions of type $A + BC \rightarrow AB + C$ had the typical rate constant $K = 10^{-11}$ cm³ s⁻¹, then $\tau_{\text{chem}}(A) = (Kn(BC))^{-1} \approx 10^4$ s to 10^8 s for $n_{\text{total}} = 10^{10}$ cm⁻³ and depending on whether the reactant BC had the abundance of CO or of a less abundant species such as CH respectively. This suggests that there will be equilibrium near the photosphere ($n_{\text{total}} \approx 10^{15}$ cm⁻³; see ref. 14) and that freezing-out will start to occur for $n_{\text{total}} \leq 10^{10}$ cm⁻³, which is about the density at grain formation in the models of Lucy¹². In the outer shell $\tau_{\text{chem}}/\tau_{\text{exp}} \propto r$ and so chemical reactions will become progressively less important. Since τ_{exp} is quite short compared with the time scales for chemical reactions in interstellar clouds¹⁵, no chemistry at all may be going on in the outer shell, and so a freeze-out model seems appropriate.

The abundances in Table 1 have been derived both from IR absorption and microwave emission lines. Because of the radio telescope beam widths involved (about 60"), we must justify the merging of these two abundance sets. For HCN and CO, Hall and Ridgway¹⁶ derive column densities $N(\text{HCN}) > 1.5 \times 10^{18}$ cm⁻², $N(\text{CO}) = 10^{20}$ cm⁻² from IR absorption lines and so $N(\text{HCN})/N(\text{CO}) > 1/67$; this agrees satisfactorily with the ratio $N(\text{HCN})/N(\text{CO}) = 1/60$ derived by Kwan and Hill¹⁷ from their model and microwave observations, and is not very different from the ratio 1/150 found by Morris *et al.*¹⁸. However, Allen and Knapp¹⁹ have recently derived $N(\text{CO}) = 4 \times 10^{17}$ cm⁻² from the $J = 2 \rightarrow 1$ ¹³CO line, in substantial agreement with the earlier figure of 2×10^{17} cm⁻² found by Wilson *et al.*⁸. These emission measurements are averages over a beam of diameter about 1'. Wannier *et al.*²⁰ report a strong radial abundance gradient for CO in IRC + 10216. If the gradient is steep enough, then a large beam will effectively dilute the observed line strengths roughly in proportion to the area of the beam. The absorption measures are with a beam whose area is about 400 times smaller and so we might expect them to lead to a column density about 400 times larger, or about 10^{20} cm⁻², as found by Hall and Ridgway¹⁶. Thus the absorption and emission measurements for CO seem to be roughly consistent. There may be difficulties if the density gradient is less steep than about $1/r^3$. We have adopted the absorption measurements in Table 1. The beam dilution effect has been allowed for by Morris¹, who used a $1/r^2$ density model in deriving column densities for SiS, SiO, CS and HC₃N, and so his emission measurements are on essentially the same scale as the absorption measurements. The value adopted for CN comes directly from the emission measurement¹⁹ of the ratio to CO; for C₂H we derived a column density from the data in ref. 21. For C₄H and C₃N we do not know in detail how the column densities were

Table 1 Molecules observed in IRC + 10216 and comparison with calculated equilibrium abundances

Molecule	Column density (cm ⁻²)				Adopted relative abundance	Expansion velocity (km s ⁻¹)	log (observed abundance / equilibrium abundance)
	Emission	Refs	Absorption	Refs			
CO	2 × 10 ¹⁷ 4 × 10 ¹⁷	8 19	5 × 10 ¹⁹ 10 ^{20*}	9 16	1	15–16	0 (by definition)
CS	1.8 × 10 ^{15*} CO/CS ≈ 10 ⁴	1 18, 42	—	—	1.8 × 10 ⁻⁵	13	+0.6
CN	10 ¹⁵ CN/CO = 2.8 × 10 ^{-3*} CN/CS ≈ 8	19 19 18, 42	—	—	2.8 × 10 ⁻³	14	+6.5
C ₃ N	10 ¹⁴ → 8 × 10 ^{14*}	22, 43	—	—	2.5 × 10 ⁻⁶	13	+0.3
HCN	CO/HCN ≈ 150 CO/HCN ≈ 60 HCN/CN ≈ 3	18 17 18, 42	≥ 1.5 × 10 ^{18*}	16	0.015	—	-0.3
HC ₃ N	1.8 × 10 ^{15*}	1	—	—	1.8 × 10 ⁻⁵	13	-0.3
HC ₅ N	4 × 10 ¹⁴	44	—	—	—	—	—
HC ₇ N	2 × 10 ¹⁴	44	—	—	—	—	—
C ₂ H	3 × 10 ¹⁴ → 2 × 10 ^{15*}	21	—	—	10 ⁻⁵	15	+0.2
C ₂ H ₂	—	—	≥ 3 × 10 ^{19*}	16, 45	0.3	—	-0.1
SiO	4.1 × 10 ^{14*}	1	—	—	4.1 × 10 ⁻⁶	10	-3.3
SiS	1.6 × 10 ^{15*}	1	—	—	1.6 × 10 ⁻⁵	13	-2.9
CH ₃ CN	Detection only	43	—	—	—	—	—
CH ₄	—	—	2.5 × 10 ^{17*}	16	2.5 × 10 ⁻³	—	-0.8
C ₄ H	4 × 10 ¹⁴ → 3 × 10 ^{15*}	22	—	—	1.2 × 10 ⁻⁵	—	-0.7
NH ₃	—	—	10 ¹⁶ –10 ^{17*}	—	10 ^{-5.5}	14	+4.0

The column densities have been measured at millimetre wavelengths in emission (radio beamwidth typically 1') and at micrometre wavelengths in absorption (IR beamwidth typically 3"). The figure for NH₃ is from Betz (personal communication and ref. 46). The adopted relative abundances are with respect to CO = 10²⁰ cm⁻² and are taken from the entries marked with an asterisk—see text. The presence of SiC in the form of grains has been inferred from an 11 μm emission feature²⁷. The equilibrium abundances correspond to log *P* = 2, log *T* = 3.1.

derived; the source is described as point-like²² and so the large-beam column densities give lower limits to the true values. For comparison with our calculations we have adopted the set of relative abundances in Table 1; we assume that they form a consistent set to within one order of magnitude.

Calculations

Chemical equilibrium calculations have been carried out for 126 molecules containing 25 elements using a program written by Clegg (described in ref. 23). The calculations cover a range of temperatures and pressures expected to occur in carbon star atmospheres, namely

$$-5 \leq \log P \leq +3, \quad 3.10 \leq \log T \leq 3.45$$

with grid spacings of 1 in log *P* and 0.05 in log *T*. Two C/O ratios were used: (1) 1.76, the averaged carbon star value used by Lucy¹² and (2) 5, the value used in calculations by Tsuji¹⁴. The O/H ratio was chosen to agree with the CO/H₂ ratio found by Kwan and Hill¹⁷ (assuming essentially all the oxygen is locked up in CO). All other elements were given solar abundances. Many of the molecules considered have dissociation energies uncertain by 0.3 eV, and so our calculations are only accurate to one order of magnitude. Molecular data were taken from Tsuji^{14,24}, updated from the JANAF tables²⁵. Two situations were considered: First with no grains present, and second with graphite grains present wherever the partial pressure *P_C* of monatomic carbon exceeds the vapour pressure *P_{v1}* of monatomic carbon over graphite; the constraint *P_C* = *P_{v1}* was imposed in those regions of the pressure–temperature plane where the supersaturation ratio *S*(C) = *P_C*/*P_{v1}* > 1 in the no-grain calculations. The results are shown in Fig. 1, in which agreement with observation is best where the density of dots is highest, in Table 1 and in Fig. 2.

Discussion

The best agreement within the range of temperature and pressure considered clearly occurs for log *T* = 3.1, log *P* = 2, with C/O = 1.76 and no grains present; Table 1 shows that the agreement in that case is remarkably good, considering the coarseness of the (*P*, *T*, C/O) grid. This suggests that most

molecules are formed in equilibrium, and that the anomalies are due to special circumstances. This result is similar to that found by Hall and Ridgway¹⁶; however, they considered only three molecules relative to CO and compared them with Tsuji's calculations¹⁴ with C/O = 5, a value for which we found much less satisfactory agreement between our calculations and observations. We confirm their conclusion that the agreement is poor if we allow graphite grains to form.

The inclusion of graphite grains by using the constraint *P_C* = *P_{v1}* gives much poorer agreement with observation. This is particularly striking for HCN and C₂H₂; in the absence of grains, reasonable agreement with observation is obtained over a wide range of *P*, *T*; when grains are added the region of agreement shrinks drastically—for C₂H₂, no *P*, *T* combination gives agreement even within an order of magnitude. This is true for C/O = 1.76 or 5.

This result is curious, since the supersaturation ratio is so large (*S* = 32 at log *P* = 2, log *T* = 3.1) that it would be very surprising if graphite grains did not form—the critical value of the ratio is usually considered to be between 2 (ionic nucleation) and 10 (heterogeneous nucleation). Also the visual extinction and IR emission clearly imply the presence of grains. A clue is afforded by the two molecules, SiS and SiO, that stand out in our equilibrium calculations as underabundant: the observed abundances are down by factors of 10³–10⁴ on the equilibrium abundances. This strongly suggests that silicon is mainly locked up in SiC (or silicate) grains in IRC + 10216, as in other carbon stars²⁶. An 11 μm feature is attributed²⁷ to SiC particles, though the absence of a 10-μm emission feature seems to rule out a significant amount of silicate grains. Morris *et al.*²⁸ discuss the low abundance of SiO in IRC + 10216 in similar terms. This suggests that, contrary to the traditional view²⁹, SiC grains form before graphite grains. There is some evidence from recent observations²⁶ that graphite forms further out than expected. Our calculations show that, for log *P* ≈ 2, SiC condenses out before C if C/O ≤ 1.1, but we were not able to obtain good agreement with observed abundances with this low C/O ratio: species containing two or more carbon atoms are much too low in abundance. At higher C/O ratios, the condensation temperatures of C and SiC are sensitive to the pressure but never differ

by more than about 200 K. Although $S(\text{C})=1$ at higher temperatures than $S(\text{SiC})=1$, we find that $S(\text{SiC})$ rises much more sharply than $S(\text{C})$ as the temperature is lowered. Given that grain formation is a highly non-equilibrium process it is quite possible that SiC grains deplete Si, and that freeze-out occurs, before carbon grains form. This conclusion seems to be strengthened when account is taken of the temperature differences between the grains and the radiation³⁰ and further work is in progress at Sussex on this aspect.

Two other molecules, NH_3 and CN, stand out as overabundant compared with equilibrium calculations, which may suggest that the freeze-out assumption is not a good one. To obtain agreement for NH_3 we would need a lower temperature and/or higher pressure while for CN we would need a higher temperature and/or lower pressure; CN is very sensitive to temperature. It is possible that both are formed on grain surfaces³¹; estimates by Turner and Gammon³² for interstellar CN give CN/CO ratios in the right range for agreement with the observed value in IRC+10216.

We did not include HC_5N and HC_7N in our calculations as no thermochemical data are available. However, Clegg (unpublished) found their abundances consistent with equilibrium predictions (within rather large errors) using estimated bond energies for these molecules. The abundances of some other large molecules including C_2H_4 , C_2H_6 , and C_6H_6 were estimated from the atomic number densities. We found that their abundances were not great enough to affect the overall calculations.

At the values of pressure and temperature that give good agreement with observation, the gas pressure is considerably higher than found in time-independent models of expanding atmospheres of cool stars. We have obtained a copy of the program used by Lucy¹² for carbon stars and computed a wide variety of models using different values of T_* (photospheric temperature), L (luminosity), M_* (mass), S_c (critical supersaturation ratio) and C/O. In all cases, the general structure of

the model is similar. Grain formation always occurs at around $\log T = 3.25$, $\log P = -3$. No point in the atmosphere is anywhere near the conditions $\log T = 3.1$, $\log P = +2$, as can be seen from Fig. 1. The same applies for the model of Menietti and Fix³³ for cool stars of more normal carbon abundance, so this result is probably generally true for cool expanding atmospheres.

However, our study of the Lucy model does show (Fig. 1) that at points before grain formation we can still obtain moderate agreement with observation for some molecules, notably HCN, HC_3N , C_2H_2 , CN, SiS and SiO, although for the first three this is mainly because of the wide range of P and T for which agreement is possible. Other molecules, CH_4 , CS, C_4H and C_3N , disagree by several orders of magnitude. In particular, the observed abundances of CH_4 and NH_3 are factors of 10^5 and 10^7 respectively higher than they would be in equilibrium in the Lucy model before grain formation; agreement with observation can only be obtained at lower temperatures. Formation of CH_4 is known to occur catalytically on grain surfaces at low temperatures³⁴ and it is therefore possible that most molecules are formed in equilibrium before grain formation and then have their abundances 'frozen-in' as the reaction rates drop with density but that CH_4 and the other saturated-bond molecule NH_3 are formed far out on grains and so do not have their equilibrium abundances. However, that would not explain the other discrepant molecules.

Furthermore, there does not seem to be a unique freeze-out point in the Lucy models—there is no single P , T point giving a best agreement with observation. Radial abundance curves were plotted for all the molecules, using the Lucy model, and it was found that some molecules agree with observation before grain formation, some after and some not at all. To explain this, it is necessary to modify the 'freeze-out' picture. Equilibrium will be a good approximation only if the reaction rates are fast enough to keep up with the changes in P , T as the atmosphere moves out. Since reaction rates are proportional to number density, we expect equilibrium to fail first for the least abundant molecules. We have made rough estimates that suggest that for at least some molecules equilibrium fails before grain formation. Ion-molecule reactions could maintain equilibrium further out, but seem unlikely to be occurring in IRC+10216, as mentioned earlier¹³. Once one molecule is out of equilibrium, all the other abundances will change and we should really do the equilibrium calculations holding fixed the abundances of those molecules that have already 'frozen out'. It would be desirable to perform a full kinetic calculation to study this problem further.

Another difficulty with the Lucy model is that the terminal velocity of the wind is considerably higher (typically 40 km s^{-1}) than observed in IRC+10216 (see Table 1). The terminal velocity v_∞ is insensitive to most parameters of the model. But a reduction in C/O to ~ 1 would reduce v_∞ sufficiently and would cut down the grain density—this could explain why we find better agreement in the absence of grains, though, as mentioned earlier, preliminary calculations seem to exclude $\text{C/O} \leq 1.1$. Alternatively, as suggested by Lucy¹² on other grounds, the grains in IRC+10216 may be larger than he assumed. The reduced grain opacity could then account for a lower v_∞ . SiC grains would also have a lower opacity in this temperature range³⁵.

An interesting test of the freeze-out model is provided by the ratio HCN/HNC . In chemical equilibrium this ratio is independent of gas pressure and abundances and only depends on the relative binding energies and the temperature (the partition functions are almost identical). The binding energy of HNC is lower than that of HCN by³⁶ 0.63 eV, from which we predict $\text{HCN}/\text{HNC} \approx 300$ at $\log T \approx 3.1$. The observed ratio of antenna temperatures is 10 (Zuckerman, personal communication) but the HCN line is optically thick. The correct ratio can be obtained from $T_A(\text{H}^{13}\text{CN})/T_A(\text{HN}^{12}\text{C})$ and scaling up by $^{12}\text{C}/^{13}\text{C} \approx 40$ (ref. 13). Since $T_A(\text{H}^{13}\text{CN}) \approx 1.3 \text{ K}$ (ref. 37) and $T_A(\text{HN}^{12}\text{C}) \approx 0.4 \text{ K}$ (Zuckerman, personal communication) we find $\text{HCN}/\text{HNC} \approx 130$, in fair agreement with our prediction.

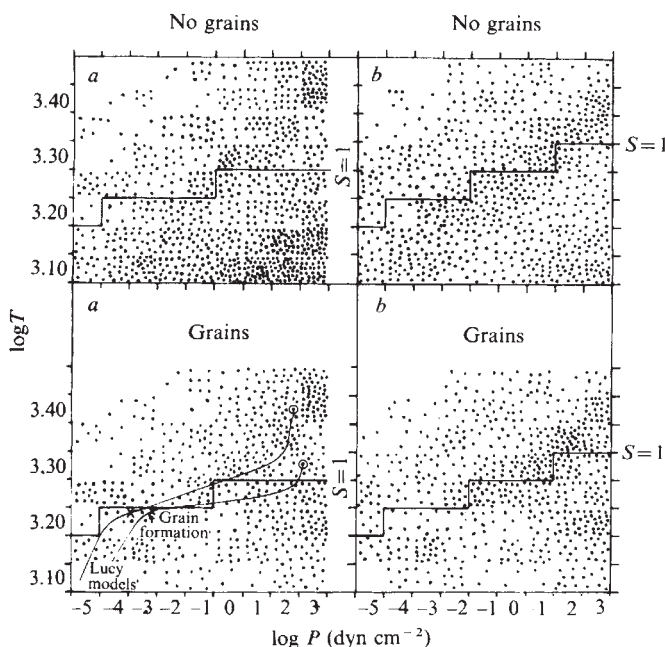


Fig. 1 A comparison of equilibrium abundances at different pressures, temperatures and C/O ratios with abundances observed in IRC+10216. *a*, C/O = 1.76; *b*, C/O = 5. The calculations were done with grid spacings of 1 in $\log P$ and 0.05 in $\log T$. Each grid point is represented on the diagram by a box and the results of the calculations are represented by dots within the box. Two dots indicate agreement for one molecule to within a factor of 10; six dots indicate agreement for one molecule to within a factor of 3. Lucy models for two different photospheric temperatures ($\log T_* = 3.40$ and 3.30) are shown. The grains and no-grains cases are identical for temperatures above $S=1$ (S is the supersaturation ratio).

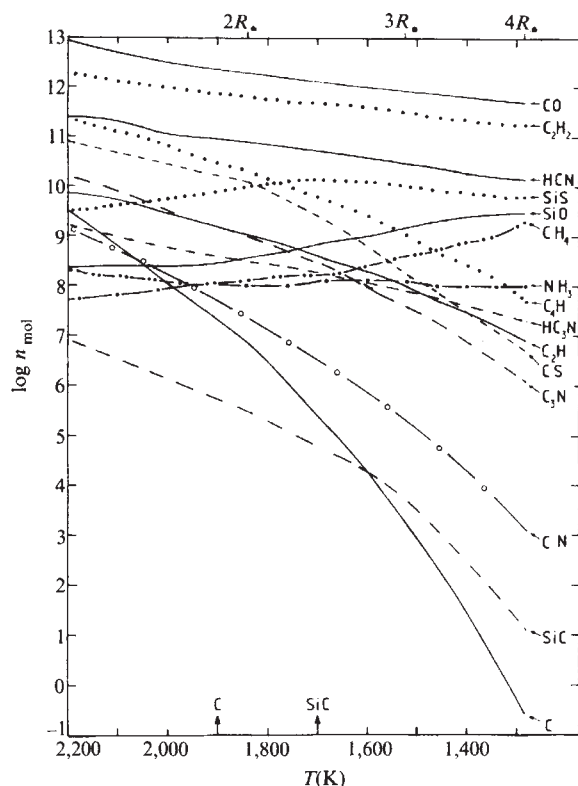


Fig. 2 Radial molecular abundance curves for chemical equilibrium based on the temperature distribution of Crabtree and Martin⁷. C/O = 1.76 and $T_* = 2,200$ K are specified and a $1/r^2$ density distribution is assumed. The temperatures at which $S(C) = 1$ and $S(SiC) = 1$ are indicated. No grain formation is allowed for.

Certainly the observed ratio is quite different from those found in many interstellar clouds³⁸, where $HCN \approx HNC$.

We have found that agreement of equilibrium calculations with observation seems to be possible only in conditions of slightly lower temperature and much higher pressure than are together consistent with conventional models of steady outflow. We have therefore sought a model in which cool compressed regions arise naturally. IRC + 10216 is known to be variable on a time scale of about 600 days³⁹ and grains and gas may well be ejected only at one phase in the cycle and so be lost in shells or 'puffs'. We speculate that this can be invoked to form the compressed regions suggested by the high pressure found for the best fit. If an inner shell absorbs radiation from the star, it shields any outer shell. The inner shell could therefore overtake the

outer shell and so perhaps compress the gas between them. This gas would also be shielded from the stellar radiation and so could cool, perhaps reaching the cool, high pressure conditions suggested by the observed molecular abundances. Close to the star there may well be a hot region, either a chromosphere or a heated shock region⁴⁰, and the dust shells would act to block UV radiation arising there. Dusty regions near such a UV source could be both a site for CN and NH_3 formation on grains^{31,32} and a sink for SiS, SiO molecules onto grains. This model predicts that molecular lines found close to the stellar surface (for example, NH_3 ; Betz, personal communication) may be variable with time.

Whether or not this model is correct, there is no doubt that the observed abundances cannot be explained in terms of freeze-out in a conventional model of the expanding atmosphere of a carbon star. The fact that we have found a good fit for two-thirds of the molecules and can suggest why the other four molecules do not fit may mean that freeze-out does occur but that actual expanding atmospheres have much higher pressures for a given temperature than does the Lucy model. That would be the case if a $1/r^2$ density dependence were valid even close to the stellar photosphere.

Future work

Our attempt to see if the 'freeze-out' model can explain the IRC + 10216 abundances has suggested some useful future lines of investigation. First, a chemical kinetics calculation for these species in a neutral, adiabatic gas flow is desirable and is underway at Texas. Even using approximate rate constants may help to identify key reactions for the destruction and formation of the observed molecules and to evaluate the accuracy of the assumption of a unique freeze-out point for all species. Second it would be useful to construct Lucy-type models of expanding atmospheres with SiC grains rather than C grains; it is hoped to do this at Sussex in the near future. On the observational side, it is important to know the radial abundance gradients of the molecules so that we can accurately match the observations²⁰. It would also be useful to have consistent measurements for all molecules, using as far as possible the same techniques and methods of reduction. Discovery of more molecules may help elucidate the chemistry in the inner shell close to the star. Finally, it would be valuable to obtain more measurements in other similar objects, such as CIT 6^{19,41}, to see whether the abundance pattern is repeated.

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Mélange in the Trondheim Nappe suggests a new tectonic model for central Norwegian Caledonides

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Extensive mélange exists near the base of the Trondheim Nappe, indicating tectonisation of syndepositionally deformed chaotic deposits. The mélange is associated with other features that are characteristic of forearc accretionary prisms. Such a tectonic setting for the Trondheim Nappe has profound implications for the evolution of the Scandinavian Caledonides.

THE Trondheim Nappe underlies a physiographic basin south of Trondheimsfjord in the central Norwegian Caledonides¹. It is the crestal plate of a pile of generally west-dipping nappes that crop out consecutively eastwards across Scandinavia². These nappes wedge out and converge to the west and seem to represent a telescoping of Lower Palaeozoic facies from eugeoclinal rocks at the top of the pile in the west to miogeoclinal-paralic strata in lower sheets to the east³. Thin slices of Precambrian basement are incorporated within and between the various allochthons.

Tectono-stratigraphic setting

The Trondheim Nappe contains a complex internal stratigraphy that has experienced several phases of penetrative deformation, at least one phase of regional metamorphism, and a complex history of thrusting. Scarcity of fossils within these strata makes correlations among the various units tenuous and speculative. This has caused confusion and controversy over stratigraphic nomenclature and structural interpretation.

The succession along the eastern margin of the Trondheim region is generally of upper greenschist-lower amphibolite facies⁴⁻⁶. Lithic similarity and stratal continuity among eastern areas indicate correlation with Wolff's⁴ key sequence (Table 1). Fossils are known only from an early Silurian graptolite locality in the Kjølhaug Group⁴, and a Dictyonema locality in uncertain relations with correlatives of the Fundsjø Group⁵. Elsewhere, strata presumed correlative with the middle of the sequence include a significant serpentinite-pebble conglomerate with early-middle Ordovician gastropods that indicate both American and European affinities⁵.

The western succession of low greenschist facies rocks in the Trondheim region reveals extreme complexity among various sedimentary and volcanic facies (P. Ryan and D. Skevington, personal communications). The nomenclature of Vogt⁷ persists for major units (Table 1), but stratigraphic details are subject to progressive revision with the discovery of new faunas. Most graptolite and shelly faunas indicate Ordovician provincialism with strong North American identity^{8,9}. The most significant aspect of the western sequence is the several kilometres-thick accumulation of spillitic basalts and pyroclastic strata that constitute the Støren Group¹⁰.

The western succession is separated everywhere from the eastern succession by a belt of unfossiliferous, higher metamorphic grade schists and gneisses assigned to the Gula Group⁴. Correlations between eastern and western successions are not only governed by the stratigraphic significance of the Gula but also depend on regional structural interpretations as well as the particular tectonic model that is favoured. For example: (1) Roberts *et al.*¹¹ have interpreted the Gula as the older core of a mushroom-shaped antiform, correlating the eastern and western sequences as outward younging inverted limbs of that antiform. (2) Gale and Roberts¹⁰ modified the mushroom model by lumping all the groups within the Trondheim Supergroup, conjectured to include various continental-margin and oceanic facies foreshortened by obduction before antiformal folding. Thus, the Gula was called the miogeoclinal equivalent of the eugeoclinal-backarc assemblage of the Hovin-Horg and Sulåmo-Slågån groups, also equivalent with the sea floor volcanics in the Støren and Fundsjø groups (assumed to be the same). (3) In contrast, Gee and Zachrisson¹² contended that the

Table 1 Major Stratigraphic units of the Trondheim Supergroup

Area	Group	Lithology	Age
Western ⁷	Horg	Calcareous sandstone, shale, quartzite conglomerate	
	U. Hovin	Tuffaceous sandstone, slate, polymict conglomerate, limestone	Caradoc-Ashgill
	L. Hovin	Graywacke, slate, felsic extrusives, andesite porphyry	Arenig-Llanvirn
	Støren	Greenstone, pillow basalt, metatuff, pyroclastic deposits	
Central	Gula	Phyllite, metagraywacke, amphibolite, biotite-sillimanite schist	?
Eastern ⁴	Slågån	Phyllite, slate, metasiltstone	
	Kjølhaug	Phyllite, metagraywacke, slate	Llandovery
	Sulåmo	Metagraywacke, phyllite, slivers of greenstone	? Mid-Ordovician
	Fundsjø	Greenstone, amphibolite, quartz keratophyre	? Tremadoc

superficially similar eastern and western successions are quite distinct and within separate nappes. The western sequence was presumed to have been deposited on the Gula, and then both thrust over the eastern sequence, which was later thrust eastwards as the Köli Nappe.

More recent interpretations are variations of the double nappe hypothesis described above^{1,2,13}, but also include extensive later translation of the Trondheim sequences/nappes farther eastward across the Baltoscandic Shield.

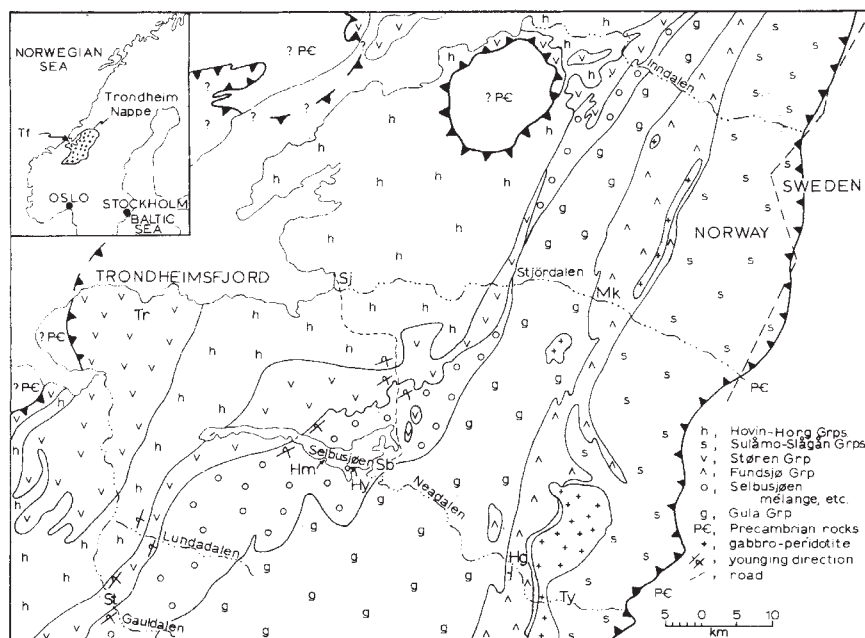


Fig. 1 Generalised geologic map of the Trondheim region, Norway (modified after Wolff¹³ and Gee²); Hg, Hegset; Hm, Hammar; Hy, Hoöya; Mk, Meråker; Sb, Selbu; Sj, Stjørdal; St, Støren; Tf, Trondheimsfjord; Tr, Trondheim; Ty, Tydal.

The resolution of these conflicting interpretations involves understanding the nature of the relationship between the Gula and adjacent volcanic groups, Støren to the west and Fjundsjø to the east. The contacts have been variously described as normal sedimentary, conjunctive-tectonic, disjunctive-tectonic, and highly tectonised with thick phyllonites and mylonites. The best exposures that reveal the Støren-Gula relationship are along the shores of Selbusjøen (Fig. 1).

The Selbusjøen mélangé

The succession north of Selbusjøen was divided into upper sedimentary (Hovin), middle volcanic (Støren), and lower sedimentary units¹⁴. The lower sedimentary unit is upper greenschist facies and is best exposed along the lake's north shore; it comprises phyllite, metagraywacke, and quartz schist. The same units extends east of Selbusjøen⁶ grading progressively into coarser and higher grade metamorphic facies typical of the Gula Group. Mélangé fabric was first recognised along the south shore of Selbusjøen and subsequently found on the north shore and in other areas.

Mélangé fabric

The most evident aspect of mélangé fabric is phyllite with fragmental debris principally of metagraywacke. Large blocks range from rounded boulders (Fig. 2) to angular phacoids; none reveal definitive evidence on origin of fragmentation. Phacoids may be boudins or pulled-apart interbeds, and boulders may have been rounded by tectonic rotation within the pervasively sheared phyllite matrix; some or all fragments also may be clasts of sedimentary origin. Less coarse debris grades through cobbles to pebbles and granules, but without apparent sorting of fragments. Some units are rich with graywacke debris, similar to pebbly mudstone (Figs 3 and 4), but even within small outcrops clast fabric ranges widely in size and angularity. In spite of deformational overprints on fragmental fabric, fragmentation seems to have been brittle in some zones (Fig. 3) and ductile in others (Fig. 4).

Volcanic material exists as lentils within the highly sheared phyllite around Selbusjøen. Discrete packets of greenstone up to several metres in length commonly lie encased within metasedimentary strata. Along the north shore of the lake apparent layers or large lentils of greenstone occur for several kilometres east of the Støren contact. The Hammar *presqu'île*

and Hoöya island (Fig. 1) consist mostly of volcanic and associated sedimentary rocks, and they may be knockers or olistoliths within the mélangé. Relationships surrounding the large volcanic masses north-east of Selbu (Fig. 1) are not clear, but an olistostrome is suggested.

Associated rocks

Associated with the mélangé of bouldery-pebbly phyllite are extensive zones of very highly sheared and crenulated phyllite with thin interbeds of metagraywacke-siltstone which have been mullioned into rods plunging easterly coincident with most other linear structures. Fragmentation in these zones certainly was tectonic, but could have occurred while the strata were relatively un lithified. Other thin zones between major lithic units are intensely tectonised into phyllonites and mylonites secondarily mineralised with quartz and sulphides. These latter zones were obviously deformed well after metamorphism.

In contrast to the penetratively deformed zones described above, other zones of weakly deformed and metamorphosed sediments also are found spatially associated with mélangé. The physical and geometric relationships of these sediments with either the mélangé or the highly sheared and mullioned zones is not clear, but contrasts in degrees of deformation and metamorphism indicate that the sediments are younger and



Fig. 2 Rounded metagraywacke boulder within pervasively sheared phyllite mélangé, roadcut along the south shore of Selbusjøen.

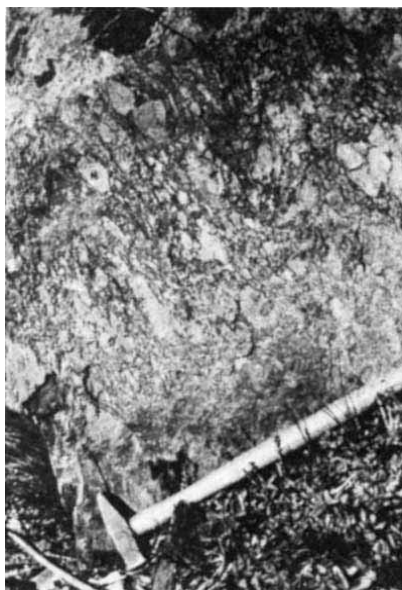


Fig. 3 Dense clast fabric of metagraywacke and volcanic debris in phyllite; note the preferred orientation of clasts, but lack of apparent flattening or stretching.

probably discordant on the more highly deformed rocks. The sedimentary strata consist of turbiditic graywacke and syndepositionally disturbed mudstone–phyllite; evidence of downslope slumping and distortion is abundant. Near the Selbu–Stjørdal road weakly deformed phyllite unconformably overlies more highly deformed quartzite–phyllite–schist retrograded from almandine grade; the unconformity cross-cuts layering in the latter rocks with minor erosional relief.

Oleson and others⁶ included all the rocks that crop out around the eastern part of Selbusjøen in the Gula Group. However, it is not known how these rocks relate to other parts of the Gula, or even if they should be included in that Group as it is used by others^{4,5}. For instance, Wolff¹⁵ implied that they were part of the Hovin Group. On the basis of graded bedding, truncated strata, and pillow lavas it is evident on both shores of Selbusjøen and along the Selbu–Stjørdal road that the Selbusjøen rocks are overturned and lie stratigraphically beneath Støren volcanics. The contact at the base of the Støren is sharp wherever exposed and seems to be primary, but it could be tectonic. The south-eastern extent of the Selbusjøen sequence is obscured by easterly gradational and continual increase in metamorphic grade up to staurolite–kyanite schists and gneiss. The latter rocks are obviously part of the Gula Group as it is used by all workers in the region.

Regional relationship

Several major valleys (-dalen) cut through the central Gula belt and provide sections across the critical contacts with adjacent units. Gauldalen cuts through the Gula Group south-east of Støren (Fig. 1), where pillow lavas indicate that the Støren Group is overturned and youngs to the north-west. The Støren–Gula contact is clearly exposed at the south-west edge of town, and seems primary with low grade metasedimentary rocks in direct contact with greenstone; graded bedding corroborates evidence from pillows indicating the metasediments underlie the volcanics. The 'upper' (north-west) part of the Gula is similar to the rocks exposed around Selbusjøen: sheared and crenulated phyllite, graphitic schist, banded metachert, weakly metamorphosed slate–graywacke, and pervasively sheared *mélange* with metagraywacke debris, all infested with hypabyssal sheets of trondhjemite. Geochemical studies of larger plutons of trondhjemite in the Støren area indicate derivation from low-K

tholeiitic basalt by partial fusion¹⁶. Five kilometres south-east of Støren, rocks of higher metamorphic grade and more typical of the Gula Group are in tectonic contact with the rocks described above; cataclasites and submylonites are present within the abrupt and narrow contact zone.

The Lundadalen section lies midway between Støren and Selbusjøen (Fig. 1) and also shows Støren Group pillow basalts to be overturned and to young westward into the exposed inverted contact with the Hovin Group of sedimentary strata replete with younging criteria. The Støren–Gula contact is not exposed, but the two groups are pervaded, respectively, with quartz keratophyre and trondhjemite sheets near the contact. A narrow zone at the western side of the Gula belt adjacent to the Støren Group consists of sheared phyllite containing both volcanic and metasedimentary debris; this zone is followed eastward by alternating belts of metagraywacke, greenschist, phyllite, metachert, amphibolite, and biotite gneiss, with numerous highly tectonised shear zones.

Neadalen extends south-east of Selbusjøen to Tydal (Fig. 1) and clearly shows the gradational increase of metamorphic grade easterly through the Gula Group. Excellent exposures in the ravine at the Hegset Dam show west-dipping pillow lavas of the Fundsjø Group to young into a sharp and steeply west-dipping fault contact with the Gula Group to the west. This is one of the few exposures of the Fundsjø Group that contains unequivocal younging direction, a point of regional controversy and tectonic significance^{3,4}. Of comparable significance is the presence of the 5 km-thick mafic layered igneous complex just east of the Fundsjø outcrop belt; the bowl-shaped body lies encased within an envelope of sheared pelitic rocks and it contains cumulate structures indicating that it is not inverted⁶.

Stjørdalen extends from Trondheimsfjord towards the Swedish border (Fig. 1); it contains the controversial section that bears on the various structural interpretations of the Trondheim region^{2,4,11,12}. This section shows a fanning of structural surfaces from east-dip at the west through vertical in the centre to gentle west-dip at the east; major tectonic wedging³ must be implicit in any model that explains the fan structure. None of the critical contacts is exposed in the Stjørdalen section. The Støren–Gula contact is a 1-km-wide tectonised zone of sheared alternating slices of metavolcanic and metasedimentary rocks of various textures and metamorphic grades. The Gula–Fundsjø contact



Fig. 4 Phyllitic pebbly mudstone with unsorted and unoriented fragmental debris of graywacke; note apparent disaggregation of block edges.

has been pervasively infested by massive sills of diorite and concordant sheets of trondhjemite.

The Inndalen section (Fig. 1) is similar to the Stjørdalen section with its fanning structure. The contacts of the Gula Group with adjacent volcanic groups are not well exposed, but are highly sheared zones pervaded with layers of diorite, trondhjemite, and quartz keratophyre. The eastern contact of the Fudsjø Group is also tectonised and followed eastward by various metasedimentary strata and zones of phyllite *mélange*, which are separated by a major strike fault from another slice of metavolcanics and the succeeding Sulåmo-Slågån Groups farther to the east.

Summarising the critical relationships among the major rock units from west to east: (1) the contact between the Støren Group and the Selbusjøen *mélange* (and associated rocks) appears to be primary; (2) the contact between the Selbusjøen rocks and rocks more typical of the Gula Group is usually tectonic, but south-east of Selbu it seems to be gradational; (3) sheets and larger bodies of trondhjemite are commonly associated with both the Selbusjøen rocks and the Gula Group; (4) at least at one locality the Fudsjø Group demonstrably youngs to the west into a fault contact with the Gula Group; (5) *mélange* is also present east of the Gula Group associated with the Fudsjø-Sulåmo contact; (6) a regional fanning of structural surfaces exists from the Hovin Group in the west to the Sulåmo Group in the east.

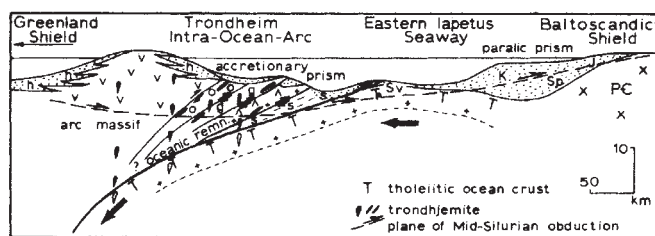


Fig. 5 Hypothetical schematic section through Early Silurian intra-ocean-arc complex within contracting Iapetus Ocean, showing relationship of various Trondheim Supergroup units to accretionary prism; J, Jämtland Supergroup; K, Köli Supergroup; Sp, Eocambrian Sparagmite; Sv, Seve Supergroup; other symbols as on Fig. 1.

The accretion model

No single criterion is exclusively indicative of any unique environment, but associations of many features are usually the best indicators of most tectono-sedimentary settings. Although *mélange* genesis is not restricted to forearc environs, *mélanges* are commonly characteristic of that tectonic setting¹⁷⁻¹⁹. Moreover, *mélanges* are often associated with other features which together indicate subduction zones and the resulting accretionary prisms¹⁹⁻²³, such as: packets of slumped upper-slope apron deposits (as seen overlying the Selbusjøen *mélange*); localised internal unconformities within accretionary basins (as near the Selbu-Stjørdal road); localised conglomerates internally derived from submarine ridges of accreted slices (as in

the Sulåmo-Slågån Groups); slices of oceanic volcanics included among the slices of accreted sediments (as within the Selbusjøen *mélange* and in the Stjørdalen section); tectonised contacts between the various accreted slices, with development of shear zones, phyllonites and mylonites (as seen in each of the sections described here); inclusion of pods and sheets of basal ophiolite sequences or layered mafic complexes and ultramafics (as near Tydal); gross fanning structure across the accretionary prism of imbricate thrusts, from low-lying slices in the front to steep wedges at the rear (as the fan structures in the Stjørdalen and Inndalen sections); anatexis of oceanic crust leading to infusion of the accretionary prisms by swarms of hypabyssal derivatives of partial fusion of tholeiitic basalt (as in the Gula Group and Selbusjøen rocks).

Figure 5 shows how a model of a forearc accretionary prism applied to the Trondheim Nappe may explain much of the structural-stratigraphic complexity in the region. The model suggests that oceanic lithosphere from an eastern Iapetus seaway was subducted westward beneath an intra-ocean-arc system consisting of low-K tholeiites and associated sublittoral deposits, which are now represented by the western succession of the Trondheim Nappe. As the eastern seaway closed through the Ordovician, slices of outer slope and abyssal sea floor deposits, and remnant slivers truncated from culminations on oceanic crusts, were accreted into and to shallow depths beneath the leading edge of the arc system; these slices are now represented by the eastern succession of the Trondheim Nappe. The central succession of the Gula Group and Selbusjøen *mélange* probably represent foreshortened inner slope and trench deposits squeezed between the arc front and accreting sea floor slices, resulting in initial penetrative deformation and metamorphism. Later evolution into the Silurian merely involves the obduction of the Ordovician intra-ocean-arc complex on to the converging Baltoscandic continent with additional deformation and regional metamorphism, and attendant thrusting of continental margin and basement slices both beneath and in advance of the obducted plate. Although the eastern Scandinavian nappes are not directly the topic of this article, the continued eastward translation of this obducted pile across Scandinavia could easily result in further imbrication and subdivision of its leading edge into the Jämtland, Köli, and Seve Nappes, with or without basal Sparagmites.

This model is admittedly speculative and at variance with all previous models. For example, the polarity of subduction advocated here is opposite to that proposed in most other models^{3,10}. Yet, I think that this new hypothesis greatly simplifies interpretations of the regional tectonic history, and that it may help solve many of the fundamental problems in the Trondheim region. It also may apply to other comparable parts of the Appalachian-Caledonian orogen, as in central Newfoundland^{24,25}.

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Experimental determination of interacting sequences in ribosomal RNA

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An approach is described for determining secondary structure in large RNA molecules, in which families of short interacting RNA fragments are isolated and identified as such by two-dimensional gel electrophoresis. Data covering over half of the 16S ribosomal RNA from Escherichia coli are presented, including two long-range interactions.

ELUCIDATION of the three-dimensional organisation of ribosomal RNA is essential to an understanding of ribosome structure and function. Unfortunately, progress in this direction has been hampered by the lack of suitably direct experimental methodology for the determination of secondary and tertiary structure in large RNA molecules. The techniques which have so far been applied to the problem include modification with single-strand-specific reagents such as kethoxal¹⁻³, binding of oligonucleotides^{4,5}, and analysis of the cutting points of ribonucleases⁶⁻⁸. All these methods, although they have yielded valuable information on the location of single-stranded regions, leave the central question essentially unanswered, namely, how are the double-stranded regions of the sequence base-paired with one another? For this it has been necessary to resort to predictions (either manual or computer-aided (for example, ref. 9)) of the sequences most likely to interact, an exercise which may well lead to totally erroneous conclusions, particularly in the case of the complex ribosomal RNA molecules, where evidence is accumulating that widely separated regions of the primary sequence are involved in mutual interactions¹⁰⁻¹³. A secondary structure prediction of the type just mentioned was made for the 16S ribosomal RNA from *Escherichia coli*⁶, but in this case the situation was even more unsatisfactory, as the model not only disagreed with much of the corresponding kethoxal data¹⁻³, but also was derived from a primary sequence which contained a large number of errors.

In this article, we present a direct experimental approach to the determination of interacting sequences in RNA, and describe its application to the 16S RNA from *E. coli*, taking advantage of the fact that the complete nucleotide sequence of this molecule has now been established^{14,15}. The method is in principle very simple. The RNA is digested with the single-strand-specific S₁ nuclease¹⁶, and the products are separated on a two-dimensional gel electrophoresis system similar to that used by Vigne *et al.* to identify two interacting fragments of 5S RNA¹⁷. The first gel dimension is run in non-denaturing conditions and the second in denaturing conditions, with the result that RNA sequences which were involved in interactions in the first dimension become dissociated and appear as pairs or families of fragments in the second dimension. The nucleotide sequences of these fragment families are then determined. The results so far have yielded information on over half of the 16S RNA molecule, and are presented in the form of a working model for the secondary structure of the regions concerned, including two long-range interactions.

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Isolation and controlled digestion of the 16S RNA

In this type of experiment, it is important to minimise perturbations of the secondary structure during isolation and digestion of the RNA. For this reason we have used a particularly mild isolation procedure in which the RNA is separated from protein on a sucrose gradient containing dodecyl sulphate, sodium chloride and magnesium acetate. Figure 1 shows, using ³H- and ¹⁴C-labelled 30S subunits, that this procedure leads to total deproteinisation of the 16S RNA, and that the RNA sediments significantly faster than a parallel sample run on our more usual gradient system containing EDTA in place of magnesium¹⁸, indicating a rather more compact conformation. For the digestion experiments, 16S RNA was prepared in the same way from ³²P-labelled 30S subunits, and was treated with S₁ nuclease at pH 5.5 in a buffer containing the same concentrations of sodium

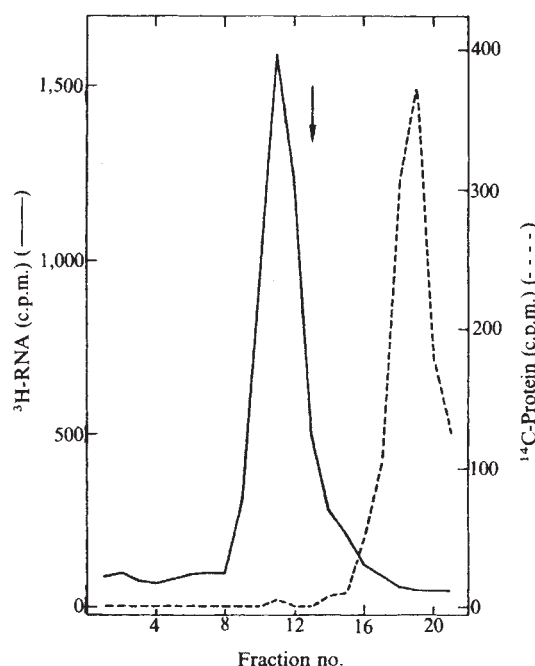


Fig. 1 Isolation of RNA. 30S subunits from *E. coli* MRE 600 were isolated as described elsewhere^{25,27}, and were applied to 7.5–30% sucrose gradients containing 0.1% SDS, 0.3 mM magnesium acetate, 10 mM sodium chloride and 10 mM Tris-HCl, pH 7.8. The gradients were run at 8 °C for 20 h at 25,000 r.p.m. The sample in this case was of 30S subunits (25 A₂₆₀ units) labelled with ³H in the RNA moiety and ¹⁴C in the proteins. Direction of sedimentation is from right to left, and the arrow denotes the position of the 16S RNA peak on a parallel gradient containing 2 mM EDTA in place of NaCl and magnesium acetate. For S₁ nuclease digestion experiments, ³²P-labelled 30S subunits (~6 A₂₆₀ units, 10⁹ c.p.m.) were applied to the gradient, and the fractions containing ³²P-RNA were pooled, precipitated with ethanol and taken up in 0.1 ml of 0.3 mM magnesium acetate, 10 mM NaCl and 2 mM Tris-HCl, pH 7.8. The solution was brought to pH 5.5 by addition of 20 mM triethanolamine acetate, pH 5.5, and was incubated for 1 h at 20 °C with S₁ nuclease (100 units per A₂₆₀ unit RNA), followed by a further hour with proteinase K (60 µg).

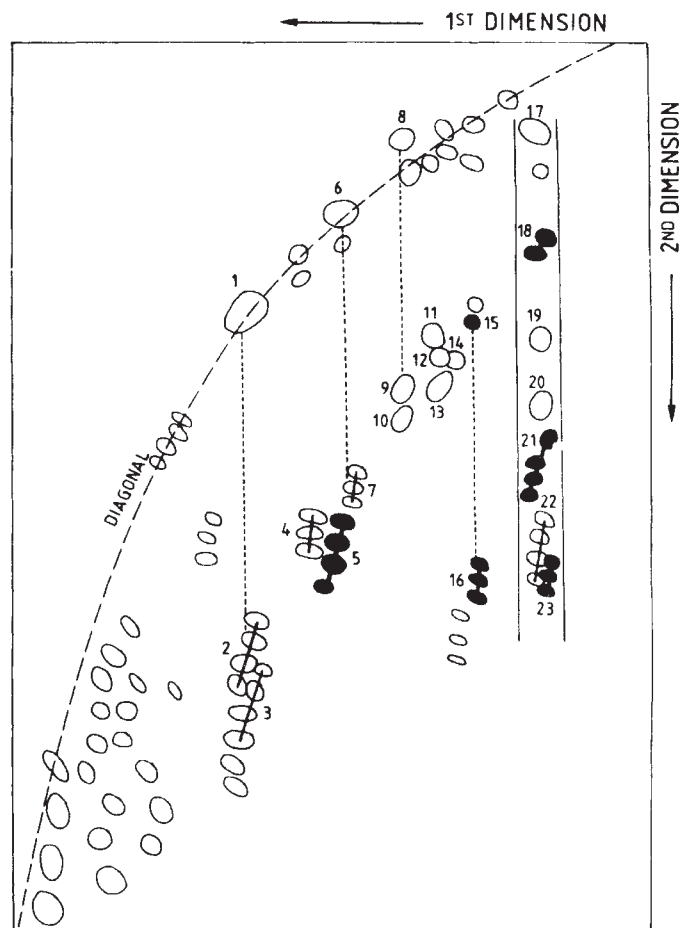


Fig. 2 Two-dimensional separation of EDTA fragments. After proteinase treatment, the S_1 -digested RNA (see Fig. 1 legend) was made 6.5 mM in EDTA and then divided into two aliquots which were incubated for 10 min at either 35 or 40 °C, respectively, followed by rapid cooling in ice. The samples were applied to a 5–20% polyacrylamide gradient slab gel (40 cm long), using the dodecyl sulphate–EDTA buffer system described in ref. 13. The gel was run at 0 °C, until a bromphenol blue marker had run 24 cm. The sample strips were autoradiographed and then applied to 20% gels containing urea, using the buffer system of ref. 28. These gels were run at room temperature, until a xylene cyanol marker had run 24 cm. Two gels (15 cm × 40 cm) were needed for each sample strip, and the figure shows the combined autoradiographs of the two gels from the 40 °C sample, with the corresponding autoradiogram from the first dimension superimposed at the top. (A substantial amount of the RNA ran as mononucleotides, not included here.) The key on the right-hand side indicates the numbers assigned to the spots or groups of spots. Groups of spots joined by bars indicate families of identical sequence, deduced from the oligonucleotide analyses (for example, the upper two spots from the fragment group 2 plus 3 contained only the fragment 2 sequence, the lower two spots only the fragment 3 sequence; the poorly resolved spots in the centre contained a mixture of both sequences 2 and 3). Spots shaded in black were either only observed or were much stronger on the gel from the 35 °C sample. Vertical lines indicate sets of related fragments.

and magnesium as were present in the sucrose gradient. Details are given in Fig. 1 legend.

Preliminary tests showed that when the digested RNA was applied to the two-dimensional gel system outlined above, the number of inter-fragment interactions was far too large to permit an analysis; in the first (non-denaturing) dimension most of the RNA ran as multi-component complexes containing many hundreds of bases. It was therefore necessary to introduce a partial denaturing treatment before applying the sample to the gel. This served to simplify the pattern of fragments by destroying most of the weaker interactions (which probably also included some isolation artefacts), leaving only the most stable structures. For this purpose, the digested RNA was first treated with proteinase K to inactivate the nuclease, and was then denatured by warming to specific temperatures in various buffers. After rapid cooling, the sample was applied to the two-dimensional gel system. (Subsequent experiments revealed that the proteinase treatment did not totally destroy the enzyme in our conditions. This fact was advantageous, in that it allowed a further mild digestion of some of the single-stranded fragments released during the denaturing incubation, resulting in a further simplification of the gel patterns; on the other hand, this same effect may be partly responsible for the ragged ends observed in the fragments (see below).)

The gel patterns obtained were very reproducible for a given denaturation treatment, but varied markedly according to the precise conditions used for this denaturation. We will describe the results of two sets of experiments, one in which the sample was denatured in the same magnesium-containing buffer as that used for the nuclease incubation, and the other in which the magnesium was removed by addition of EDTA. The sets of fragments will be referred to as 'magnesium' and 'EDTA' fragments, respectively.

Fragments partially denatured in the presence of EDTA

Figure 2 shows the two-dimensional gel pattern obtained from an S_1 digest of 16S RNA, partially denatured in the presence of EDTA. Precise conditions for the sample preparation and gel electrophoresis are given in the legend. Denaturation was made at either 35 or 40 °C, and the key diagram on the right of Fig. 2 indicates the additional spots which were observed at the lower temperature, as well as those which were present at both temperatures. The figure shows the expected properties, namely, a 'diagonal' consisting of fragments either already denatured or containing uncut loops of secondary structure,

together with clear families of fragments lying below this diagonal. (The diagonal is curved as a result of the gradient gel system used for the first dimension.)

³²P-labelled RNA fragments were extracted from the gels, digested with T₁ ribonuclease and subjected to oligonucleotide analysis by the classical techniques of Sanger *et al.*¹⁹. The gel separation was sufficiently good and the background sufficiently low for clean fingerprint data to be obtained in most cases without further purification, and a typical fingerprint (bases 577–615 in the 16S sequence¹⁴) is shown in Fig. 3. Molarities of the oligonucleotides present were determined from the fingerprint, and their identities were confirmed by digestion with ribonuclease A as described in Fig. 3 legend. The data were then fitted to the 16S sequence of Brosius *et al.*¹⁴, and the fragment analyses from the gels corresponding to both denaturation temperatures (35 and 40°C) were combined. From these analyses, a picture of the gel pattern in terms of nucleotide sequences could be built up, fragments of identical sequence (but differing in steps of one nucleotide in length) being indicated by the bars joining the spots in Fig. 2. The location in 16S RNA of the sequence corresponding to each numbered spot or group of spots from Fig. 2 is presented in Fig. 5. It is clear from the groups of related spots in Fig. 2 that in many cases the fragments had 'ragged ends', and in addition it was not always possible to localise the 5' and 3' ends of the fragments precisely, either as a result of the particular region of the 16S sequence concerned (such as a fragment ending in a run of several G residues), or due to an ambiguity in the identification of the 5' and 3' oligonucleotides (which are of the type pXpYpZp and XpYpZ, respectively, after consecutive S₁ and T₁ digestion). Thus, the 5' and 3' ends of the fragments as depicted in Fig. 5 should be taken as being approximate to the extent of ± 2 bases. In all cases, however, there was excellent agreement between the fingerprint data and the length of the fragment as estimated by its mobility in the second-dimension gel.

Comparison of the data of Figs 2 and 5 shows that the expected fragment relationships appear. For example, fragment 1 (occurring about 600 nucleotides from the 5' end of 16S RNA) is the sum of fragments 2 and 3, but the latter are not quite contiguous sequences, being separated by a gap of several nucleotides. Further up the gel, a similar situation is found with the larger fragments 6 and 7, from the same region of the RNA, except that in this case (in contrast to spots 2 and 3) the spots containing fragment 7 were a mixture of both halves (denoted 7 and 7' in Fig. 5) of the sequence contained in fragment 6, again with a gap in the middle. Thus, fragments 2–3 and 7–7' must be involved in base-paired interactions with one another, and run in the first dimension together with the corresponding 'uncut' loop fragments 1 and 6. The overall pattern of fragments from Fig. 2 will be discussed below, together with the results from the set of magnesium fragments.

Fragments partially denatured in the presence of magnesium

Figure 4 shows the two-dimensional gel pattern obtained after denaturation at 45 or 50°C in the presence of magnesium. A different gel system was used for the first dimension in this case (see Fig. 4 legend) to keep magnesium present as far as the second dimension, and the acrylamide concentration was also increased to improve the resolution of the smaller fragments. In other respects, Fig. 4 should be interpreted in exactly the same way as Fig. 2. The pattern of fragments is obviously rather more complex in this case, and in some parts of the gel (such as the spots between nos 28 and 29) the fingerprints obtained were mixtures which could not be clearly interpreted. Again, gels corresponding to both denaturation temperatures were analysed, but in this case, for simplicity, the spots on or near the diagonal (with the exception of nos 42 and 48) were not subjected to fingerprinting. It is interesting that RNA prepared by

the standard phenol–dodecyl sulphate technique or by acetic acid–urea treatment²⁰ gave identical gel patterns to that of Fig. 2 after digestion followed by denaturation in EDTA. However, when the digest was denatured in the presence of magnesium, RNA prepared by either of these methods gave patterns resembling that of Fig. 2 more closely than that of Fig. 4.

The results of all the analyses from the gel of Fig. 4 are included in Fig. 5, and the structures which were deduced for the interacting fragments are presented in Fig. 6. Figures 2, 4, 5 and 6 should be considered together in the following discussion, fragments 1–23 arising from the gel of Fig. 2, and 24–50 from that of Fig. 4.

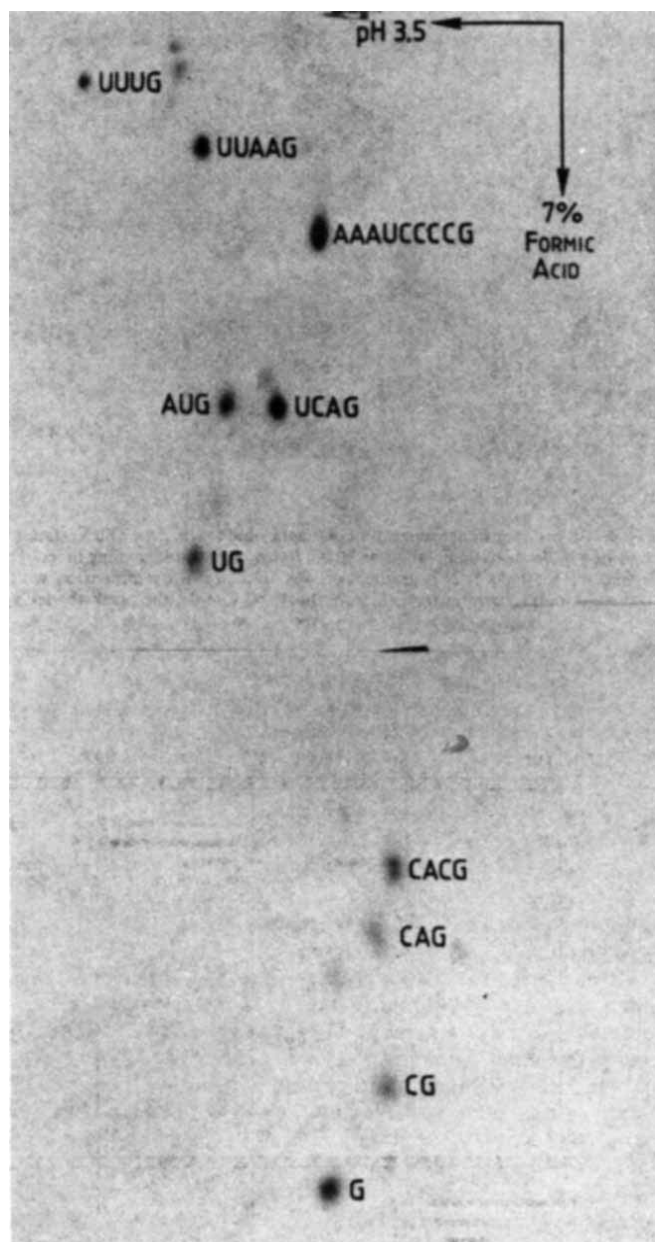
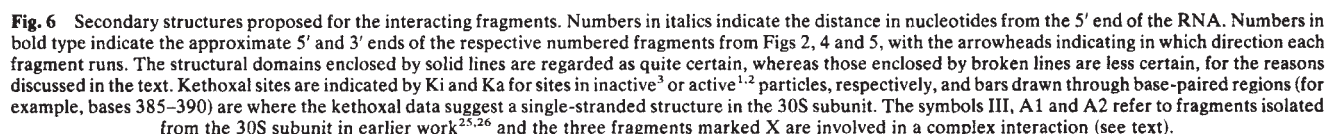


Fig. 3 Example of a T₁ ribonuclease fingerprint. ³²P-labelled spots were extracted from the two-dimensional gels (Figs 2 or 4) with phenol and dodecyl sulphate buffer as described elsewhere²⁵. Each RNA sample was precipitated from the aqueous layer with ethanol, in the presence of 50 µg carrier RNA, and, after a further ethanol precipitation, was dissolved in 10 µl of 10 mM Tris-HCl, pH 7.8, and 1 mM EDTA, and digested with T₁ ribonuclease. Oligonucleotide analysis was made according to the methods of Sanger *et al.*¹⁹ as described elsewhere²⁵. The spots were cut from the fingerprints, analysed for radioactivity and subjected to secondary digestion with ribonuclease A as described elsewhere²⁹, using the two-dimensional separation system of ref. 30. The fingerprint in this case is of bases 577–615 of the 16S RNA sequence¹⁴.



The simplest case to consider is the set of fragments between bases 400 and 500 (Fig. 5), consisting of the fragment pairs 29-29', 34-35 and 36-37. This set forms a clear 'pyramid' of fragments of increasing length, which can be drawn as the rather symmetrical structure shown in Fig. 6, remembering (as already mentioned) that the 5' and 3' ends of the fragments were not always precisely definable. Fragments corresponding to the shortest base-paired regions (bases 450-460 and 465-475) were not found; such small fragments would have run on the gel below fragments 24-27 (Fig. 4), an area which was too complex to

interpret. In this context, remember also that S_1 nuclease does not attack all apparently single-stranded regions in RNA (for example, in tRNA only the anticodon loop is cut²¹).

Immediately adjacent to the structure just discussed, there is a strong long-range interaction of 15 base pairs, defined by the fragment mixture 28–28'. The structure is assigned as tentative in Fig. 6 because, although the interaction was reproducibly detected and the fingerprint data were clear, the sequences concerned have not so far been observed in larger fragment structures where each component of the interaction could be observed separately. In this case, the kethoxal sites suggest that the weaker half of the structure is opened up in the 30S subunit; such a situation would be consistent with the finding that the RNA has a lower helical content in the subunits than in the isolated form²².

The structures drawn for the interactions in the regions between bases 100–300 and 800–900 are also assigned as tentative. In the latter case, the interacting fragments 12 and 11 or 13 were reproducibly found, but the presence of fragment 14 without a 'partner' (Fig. 2) suggests that the 5' half of this loop (fragment 12) is easily digested to smaller pieces which were not analysed in the experiment of Fig. 2. The structure proposed for this interaction has a single-stranded 'tail' at the 5' end (again suggesting a missing piece), this arrangement being supported by the finding of precisely this tail as an unpartnered fragment (no. 27) in Fig. 4. In the region of bases 100–300, a simple loop structure could be drawn for bases 231–296, containing the interacting fragments 5 and 5' (Fig. 2). However, the sequence of fragment 5' overlaps that found for fragment 31 (Fig. 4) by several bases, the latter fragment clearly interacting with fragment 30. An interaction similar to that between 5 and 5' was nevertheless also present in the gel of Fig. 4, as evidenced by spot 32, whose fingerprint indicated a mixture of the sequences contained in fragments 5, 5' and 31. In this context, the significance of the small fragments (24, 25 and 26, Fig. 4), which overlap fragment 31 and extend it in the 5' direction, is not clear.

The most detailed information was obtained from the region of bases 550–800. For simplicity, only selected fragments are indicated in Fig. 6, and the reader should refer to Fig. 5 for fragments of similar or identical sequence (for example, fragment 15 is the same as nos 19, 44, 49 and 38). As already mentioned, the interacting fragments 2–3 and 7–7' clearly define the loop structure of bases 587–653. The next pair of fragments (9 and 10) can be drawn as a simple base-paired extension to this structure, but the families of larger fragments contradict this simple extension. Fragments 44, 45 and 46 (Fig. 4) reproducibly showed the presence of an extra piece of RNA (bases 733–764), which must be involved in the structure, and fragments 49, 49' and 50 indicate a further elongation in the extreme 5' and 3' directions. The interaction between the three fragments is accordingly represented by the structure shown in Fig. 6, and other minor components of the gels from this RNA region are consistent with the structure. For example, fragments 39, 40 and 41 appear as a family in which both the left and right ends of the structure are missing (Fig. 6), and fragments 42 and 48 correspond to uncut loops running approximately from bases 570 to 670. Fragments 15 and 16 (Fig. 2) are also consistent, although here a piece of RNA must be 'missing' from the analysis to account for the mobility of the complex in the first gel dimension. Note also that this structure agrees exactly with the secondary structure of part of this region proposed by Müller *et al.* for the RNA binding site of proteins S8 and S15 (ref. 23).

Fragments 44, 45 and 46 (Fig. 4) were identical to fragments 19, 20 and 23 (Fig. 2), respectively. In the latter case, fragment 23 disappeared at the higher temperature (40 °C, Fig. 2), allowing the complex between 19 and 20 to run slightly faster in the first dimension. This enabled the complicated region at the right-hand side of Fig. 2 to be resolved, as it follows that fragments 19, 20 and 23 form one family, and 17, 18, 21 and 22 another (Fig. 5). These fragments suggested the long-range interaction between bases ~920–940 and 1,380–1,400, an

interaction which was very precisely and reproducibly confirmed by fragments 4 and 4' (Fig. 2). This is probably the same interaction as that proposed (but not localised) by Ehresmann *et al.*²⁴, although as with the other long-range interaction already discussed the distribution of kethoxal sites suggests that the weaker ends of the base-paired structure are open in the ribosome. This structure also fits well with the 5' and 3' ends of the RNA from ribonucleoprotein 'fragment III', isolated from the 30S subunit²⁵, and encompasses a complex interaction between the RNA fragments named 'X' from this region. The latter fragments were found in earlier experiments, but can be arranged together in too many different ways to merit drawing a structure at this stage. The remainder of the region (bases 1,400–1,500) can be drawn as a simple loop structure, fragment 17 (lying just below the diagonal) representing the uncut loop together with the tail interacting with fragment 22, whereas fragments 18 and 21 represent the corresponding loop halves, which disappeared at the higher temperature (Fig. 2). This structure also agrees well with the sequences of two RNA fragments (denoted A1 and A2) which were isolated from the 30S subunit²⁶.

Conclusion

The experiments described here offer a basis for determining the secondary structure of ribosomal RNA. Although the data were obtained from isolated RNA, in general they are in good agreement with other data from the 30S subunit, in such cases where a comparison is possible. There are, however, some anomalies, suggesting structural heterogeneities in the RNA. Such heterogeneities could reflect multiple coexisting structures *in vivo*, or may represent artefacts introduced by the isolation procedure. Experiments now in progress, using the 30S subunit as starting material as well as RNA isolated by various methods, should help to resolve this aspect of the problem. At the same time it cannot yet be entirely excluded that some conformational rearrangements occur during the partial denaturation treatment of the digests, but this should become clearer as the data accumulate. We believe that the further development and application of this approach should lead not only to a reasonably accurate experimental model of the ribosomal RNA structure both within the subunits and as an isolated moiety, but also to an understanding of the relative stabilities of the individual parts of the structure and their dependence on ionic environment.

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Two-step binding of eukaryotic ribosomes to brome mosaic virus RNA3

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Although brome mosaic virus RNA3 has only one translatable cistron, it can bind two 80S ribosomes at initiation. One ribosome binds at the first AUG codon (bases 92–94). The other binds nearer the 5' end at an entry or holding site. Disome formation is thus unrelated to a silent cistron ~1,000 bases downstream.

ALTHOUGH the messenger-sense genomic RNAs of many viruses infectious to eukaryotes encode several cistrons, only the 5' proximal cistron of each such RNA is translatable¹. Translation in eukaryotes is generally considered to be restricted to 5' terminal cistrons by initiation requirements for a distinctive RNA sequence and a 5' terminal blocking group—the cap. However, sequences preceding coding regions show no obvious common features² and the cap itself is dispensable³. Removal of the cap reduces the rate but not the specificity of initiation⁴, and several viral mRNAs have been found that are not capped. Moreover, eukaryotic ribosomes are capable of recognising internal cistrons on uncapped prokaryotic mRNAs⁵, although it is possible that such RNAs may have been modified by enzymes existing in the cell-free extracts. The characteristics that define a functional eukaryotic ribosome binding site and prevent such a site from occurring internally in the RNA thus remain undetermined.

We have examined these issues as they apply to the brome mosaic virus (BMV) RNAs. BMV is a small, icosahedral virus of cereal plants. Much is known about the location of the BMV coding regions and their translation. The BMV genome consists of three distinct RNA molecules (designated RNA1, RNA2 and RNA3 in decreasing order of size) encapsidated in three virions⁶. These RNAs are necessary and sufficient for infectivity, but virions containing RNA3 (2,200 bases) also contain one copy of a subgenomic RNA designated RNA4 (900 bases). RNA3 contains the nucleotide sequences and genetic information of RNA4, and can act as a precursor to RNA4 found in progeny viruses resulting from infection with RNAs 1, 2 and 3 alone. *In vitro* translation experiments show that RNA4, although dispensable for infectivity, is the functional mRNA for the viral coat protein. The only translation product detectable when BMV RNA3 is used as a template in optimal conditions is a polypeptide (MW 35,000) unrelated to coat protein (MW 20,000)⁷. Thus RNA3 contains at least two cistrons—an accessible cistron and a coat protein cistron which for some reason is inactive. Subsequent experience has shown that similar genomic RNAs containing one or more untranslatable coding regions, plus their accompanying subgenomic RNAs, which are active messengers, are common in viral systems¹. The untranslatable coding regions of these genomic RNAs are now popularly called silent cistrons.

During our experiments we observed that, in cell-free extracts of wheat embryo, two ribosomes can bind to RNA3 in conditions where initiation can proceed but elongation is inhibited. In identical conditions RNA4 binds only monosomes. Originally it

was considered possible that the binding of two ribosomes was related to the dicistronic organisation of BMV RNA3. However, BMV RNAs 1 and 2 also bound two ribosomes and they, as well as RNA4, are thought to be monocistronic messengers⁷. In fact, the binding of two ribosomes in similar circumstances has been observed with several viral RNAs including tobacco mosaic virus genomic RNA⁸, alfalfa mosaic virus RNA3⁹, and encephalomyocarditis virus RNA (our observation). As these RNAs differ considerably in their organisation and structural features, it seemed likely that some rather subtle structural feature common to all these viral RNAs was responsible for disome formation, and that this might be important both to the reproduction of the virus and to the mechanism of protein synthesis. Accordingly, we studied ribosome binding to RNA3 using procedures designed to reveal features of the nucleotide sequence involved in disome formation.

Ribosomal binding to RNA3 and to 5' terminal fragments of RNA3

Two ribosomes bound to one molecule of ³²P-labelled BMV RNA3 when translational elongation was blocked by sparsomycin¹⁰ anisomycin¹¹ or T2 toxin¹² used singly or in combination (Fig. 1a). (T2 toxin, a derivative of 12,13-epoxytrichothecene, inhibits the formation of the first dipeptide bond but has no effect on the formation of 80S initiation complexes.) In all cases in which ribosomes were present in excess, the amount of labelled RNA present in structures sedimenting at the rate of disomes exceeded that sedimenting as monosomes by at least a factor of two. In Fig. 1a the smaller peak of radioactive RNA coincided with the peak of UV absorbance due to 80S ribosomes. The larger peak co-sedimented with the disome peak from polysome profiles run in parallel gradients. We observed similar patterns with BMV RNAs 1 and 2 (data not shown), but not with BMV RNA4 (Fig. 1b), which presented only a single peak of RNA radioactivity associated with monosomes.

Although the binding experiments above were done with uniformly labelled RNA, for ease in correlation with sequence analysis it is convenient to use terminally-labelled RNA. We chose to label BMV RNA3 at its 5' end with the capping enzyme from vaccinia virus cores described by Moss and co-workers¹⁴. This enzyme transfers a GMP moiety from GTP to 5'-diphosphorylated RNA termini and then, in the presence of S-adenosyl methionine, methylates the resulting inverted G residue and the 5' terminal ribose of the chain proper. When partially purified, the enzyme is associated with a 5'-triphosphate γ -phosphatase. It has the important feature that it regenerates the natural 5' terminal configuration of capped messenger RNA. The reconstructed 5' end does have a ribose methylation lacking in the original BMV RNA, but this has been shown to have little effect on protein synthesis¹⁶. The enzyme has three other advantages over polynucleotide kinase, the enzyme most frequently used for 5' labelling RNA. First, despite the fact that decapped and dephosphorylated BMV RNA3, like some other eukaryotic mRNAs¹⁷, is an extremely poor substrate for polynucleotide

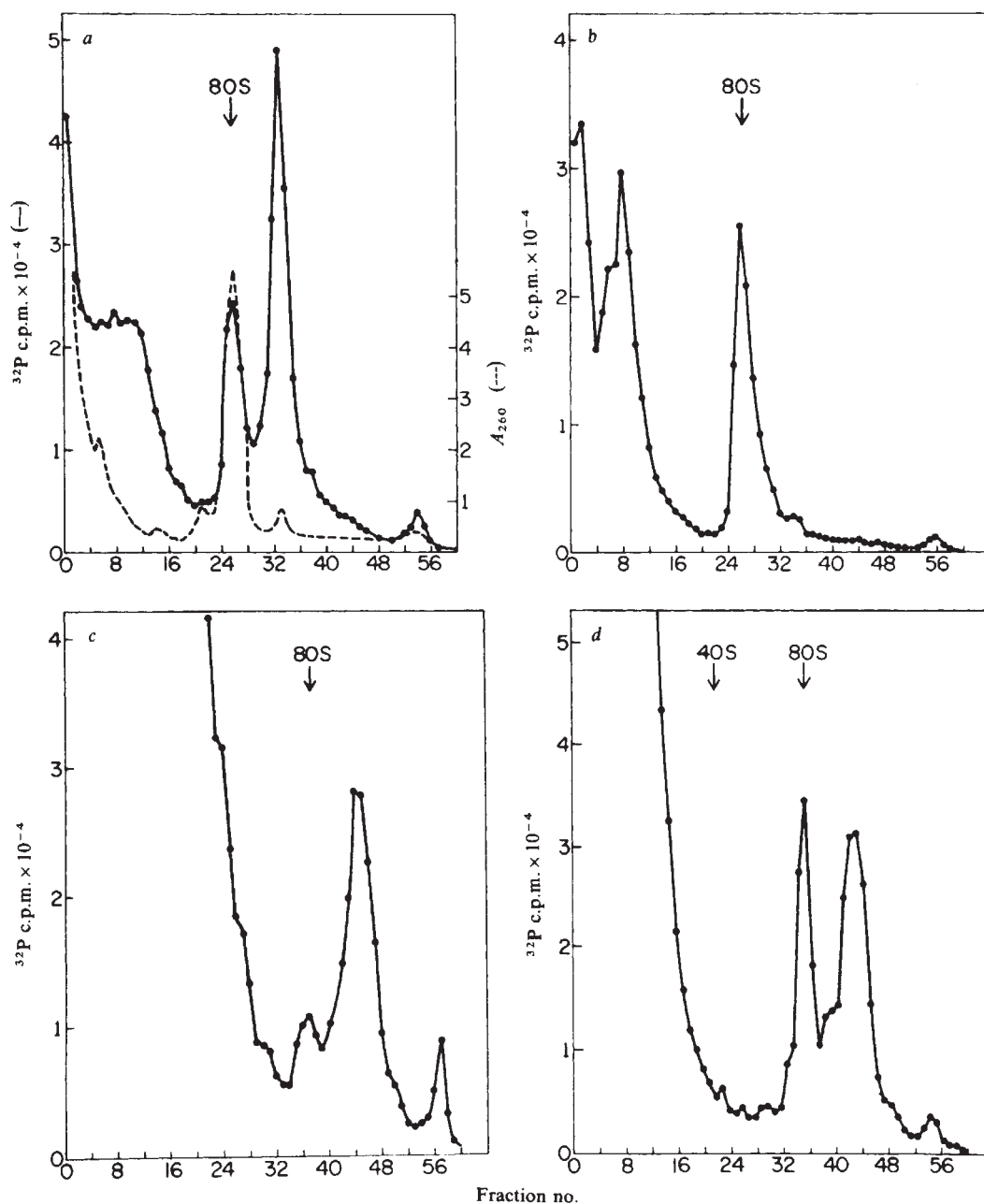


Fig. 1 Detection of the binding of two ribosomes to RNA3 by sucrose density gradient sedimentation analysis. Preparation of wheat embryo extracts and ^{32}P -uniformly labelled BMV RNA components were as previously described¹³. Terminal labelling with the vaccinia capping enzyme¹⁴ was carried out in a 50 μl reaction mix containing 20 μg decapped BMV RNA3, 20 μM [α - ^{32}P]GTP (480 Ci mmol^{-1} , Amersham), 0.16 mM ATP¹⁵, 50 μM *S*-adenosyl methionine, 50 mM Tris-Cl pH 7.8, 1 mM MgCl_2 , 1 mM DTT and 5 μl of DEAE-cellulose purified enzyme preparation. The reaction mix was incubated for 15 min at 37 $^\circ\text{C}$, phenol extracted twice, ether extracted twice, and ethanol precipitated. Conditions for ribosome binding reactions (100 μl) were as described elsewhere¹³ except that the creatine phosphokinase concentration was 20 $\mu\text{g ml}^{-1}$ instead of 125 $\mu\text{g ml}^{-1}$. The reaction mixtures were incubated with 0.45 mM anisomycin plus 0.06 mM T2 toxin (a and b) or with 0.2 mM sparsomycin (c and d) for 5 min followed by addition of ^{32}P -labelled RNAs and further incubated for 10 min. The reaction mixtures were cooled on ice and applied onto 10–40% sucrose density gradients prepared 50 mM Tris-HCl, pH 7.6, 50 mM KCl and 4 mM Mg-acetate. The gradients were centrifuged in a Spinco SW41 rotor at 36,000 r.p.m. for 3 (a and b) or 4 h (c and d) and fractionated with an ISCO gradient fractionator. a, 5 μg ^{32}P -uniformly labelled RNA3; b, 10 μg ^{32}P -uniformly labelled RNA4; c, 5 μg ^{32}P -terminally labelled RNA3; d, 5 μg ^{32}P -terminally labelled RNA3, partially digested with RNase T₁ as described in Fig. 2, phenol and ether extracted, and ethanol precipitated before ribosome binding. The increase in ^{32}P counts at the top of the gradients shown in c and d compared to a and b is due to the presence of unincorporated [α - ^{32}P]GTP from the labelling reaction.

kinase, the vaccinia enzyme consistently gave high incorporations of ^{32}P . Second, even our partially purified preparation was comparatively free of RNase. Third, as the enzyme recognises only 5' di- or tri-phosphorylated ends, it does not label internal nicks in the RNA. Figure 1c shows that BMV RNA3, decapped by periodate oxidation and β -elimination, then recapped with the vaccinia capping enzyme and [α - ^{32}P]GTP in the presence of *S*-adenosyl methionine, binds to wheat embryo ribosomes in conditions of sparsomycin inhibition in a manner similar to that of uniformly labelled RNA3.

Fragments of terminally-labelled BMV RNA3, partially digested with nucleases, also bind two ribosomes in the presence of sparsomycin. Figure 1d shows the binding pattern of a partial RNase T₁ digest of 5' labelled, capped and methylated RNA3 in conditions of sparsomycin inhibition. Partial RNase ϕ 1 digests show a similar pattern. Preliminary experiments showed that the

size of these fragments was well within the range of high-resolution sequencing gels, so in further experiments we used partial digestion conditions that gave relatively uniform cleavage of every susceptible site over the first few hundred residues and allowed us to pinpoint the parts of the RNA responsible for binding. For detailed analysis of these experiments we needed to determine the 5' terminal sequence of RNA3.

5' Terminal sequence of BMV RNA3

The sequence of the first 164 residues beyond the capping group $\text{m}^7\text{G}^5\text{ppp}^5\text{G}$ was determined by rapid gel methods using base-specific ribonucleases^{18,19} as shown in Fig. 2. We determined the first few residues from the 5' terminus independently by partial digestion with nuclease P₁ followed by mobility shift analysis

using two dimensional cellulose acetate electrophoresis and homochromatography²¹ to allow unambiguous counting of the bases and to confirm the sequence of the extreme 5' end. The sequence is shown in Fig. 3.

On initial sequencing gels, pyrimidine assignments were made by examining the RNase $\phi 1$ cleavage pattern on each side of the base in question. Contrary to the published dinucleoside hydrolysis frequencies²² but in agreement with other observations²³ we found that some A—C bonds (those between residues 12–13, 53–54, 99–100, 131–132, and 141–142) were resistant to RNase $\phi 1$ cleavage in our conditions. In later gel sequencing experiments RNase Phy M was used to distinguish between C and U residues. RNase Phy M, which cleaves after A and U residues (H. Donis-Keller, personal communication), has a much simpler

base specificity than RNase $\phi 1$ and thus provides a more accurately interpretable cleavage pattern.

RNA fragments associated with monosomes and disomes

5' End-labelled RNA fragments recovered from the monosome and disome regions of sucrose gradients such as that shown in Fig. 1d were freed of protein by phenol extraction and electrophoresed on a sequencing gel in parallel with samples of the original digests that had not been bound to ribosomes (Fig. 4, lanes c–h). As a second set of controls, full length RNA3 was recovered from disomes and then digested and electrophoresed

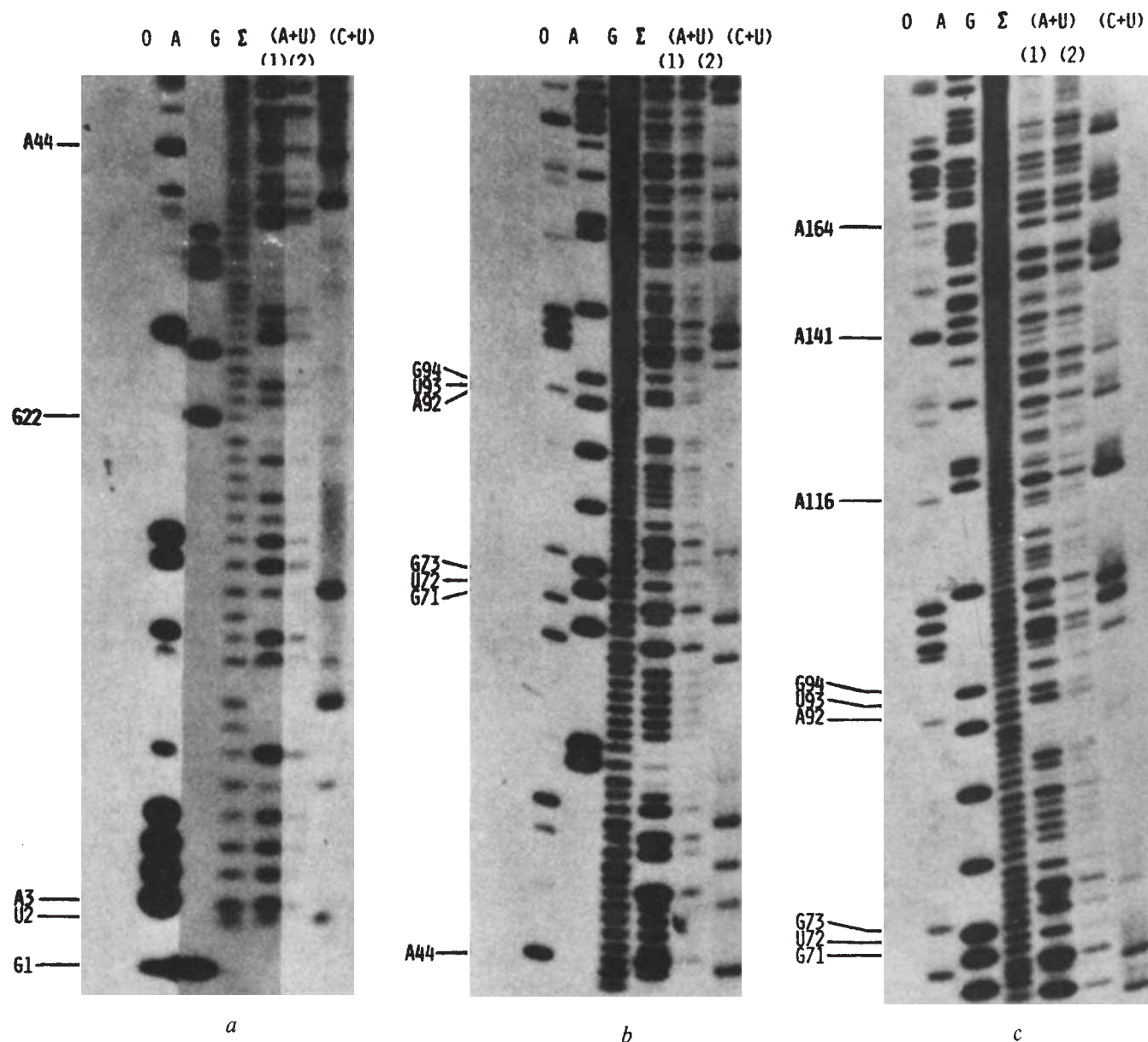


Fig. 2 Sequencing gels of 5'-labelled BMV RNA3. BMV RNA3 was 5'-labelled as described in Fig. 1, except that S-adenosyl methionine was omitted. Full-length labelled RNA was isolated on a 5% acrylamide 7 M urea slab gel. Enzyme digests and a minus enzyme control each contained 2.5 μ g RNA in 10 μ l 20 mM Na-citrate pH 5.0, 1 mM EDTA, 7 M urea (ultrapure urea solutions were deionised with Fisher Rextyn I-300 resin before use). Digests were incubated 15 min at 50 °C, after which 2.5 μ l of 0.3% xylene cyanol, bromphenol blue, 10 mM EDTA in formamide was added to each. Two different strength digests were done for each enzyme. For RNases U2, T1, and pancreatic RNase, like enzyme digests were mixed together after incubation. For formamide digestion 5 μ g of RNA in 20 μ l 90% formamide, 10% double-distilled H₂O was incubated 45 min at 100 °C. An aliquot of 2 μ l formamide/dye mix was then added. Samples were heated for 1 min at 100 °C and 2 μ l aliquots of each were loaded onto freshly polymerised 20 $\times$ 40 $\times$ 0.035 cm 7 M urea slab gels²⁰ and electrophoresed at 30–35 mA in 87.5 mM Tris-borate pH 8.3, 2.75 mM EDTA. a, Shows a 12% acrylamide gel and b and c show 8% acrylamide gels. Gels were autoradiographed at –70 °C with an unflashed Kodak X-Omat R film clamped tightly between the gel and a Dupont Cronex Lightning Plus intensifying screen. The following symbols are used to designate the various lanes: O, minus enzyme control; A, 2.0, 0.2 units RNase U2 (Calbiochem) per μ g RNA; G, 0.04, 0.004 units RNase T1 (Calbiochem) per μ g RNA; Σ , formamide digest; A + U(1), 0.08 arbitrary units RNase Phy M per μ g RNA; A + U(2), 0.008 arbitrary units RNase Phy M per μ g RNA; C + U, 2×10^{-4} , 2×10^{-5} μ g pancreatic RNase (Worthington) per μ g RNA.

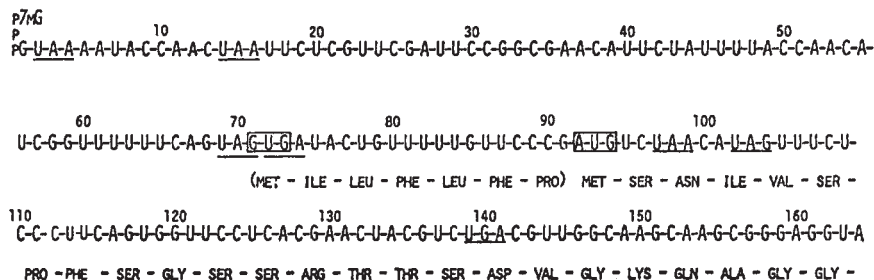


Fig. 3 The nucleotide sequence of the 5' terminal 164 bases of BMV RNA3. The first AUG and GUG codons from the 5' end are boxed and nonsense codons are underlined. The amino acid sequence of the protein which would result from initiation at the first AUG is aligned with the nucleotide sequence. Extra N-terminal amino acids which would result from initiation at the first GUG are shown in parentheses.

(Fig. 4, lanes *a* and *b*). The resulting band patterns correspond exactly to those obtained by digestion of RNA3 not exposed to wheat embryo extracts (Fig. 4, lanes *e* and *h*, or Fig. 2), thus excluding the possibility that the ribosome binding behaviour we observe is an artefact of sequence rearrangement (that is, RNA splicing) in the wheat embryo extracts.

A distinct difference in length distribution was observed between RNA recovered from monosomes (Fig. 4, lanes *d* and *g*) and that recovered from disomes (Fig. 4, lanes *c* and *f*): the monosome peak contained fragments 29 to 91 residues long while the disome peak contained fragments 94 residues and

longer, as well as faint traces of fragments 69 to 91 residues long (see Fig. 4 legend). The striking transition in ribosome binding properties occurring with fragments of 94 bases or longer is clearly seen on the autoradiogram as a lateral switch in the band pattern from the monosome to disome lanes.

The fact that RNA3 can bind two ribosomes efficiently, but no more than two when translation is inhibited implies that RNA3 has two sites that interact strongly with ribosomes. Efficient disome formation with 5' terminal fragments of RNA3 as short as 94 residues and failure of shorter fragments to bind two ribosomes implies that one site is near base 94. Bases 92–94 comprise the first 5' proximal AUG codon, which is presumably an initiation codon for protein synthesis. These bases are followed by an open reading frame as far as the known sequence extends; the other two reading frames are closed by nonsense codons following almost immediately after the AUG. The second ribosome on 94 long fragments, and thus presumably on longer fragments and on intact RNA, must be on the 5' side of the AUG codon. As fragments 29 residues long are recovered from monosomes and shorter fragments are not, the region around base 29 must have the potential for interaction with ribosomes. However, some fragments in the range of 35 to 60 residues long are recovered from monosomes only in poor yield, possibly because the base 29 binding site may be buried by RNA secondary structure in fragments 35 residues long and longer (see below). Another potential site for ribosome interaction exists around base 70 corresponding to fragments which are again recovered from monosomes in good yield. This site would be in the vicinity of the first GUG codon at bases 71–73. The failure to form trisomes indicates, however, that on intact RNA3 stable ribosome binding does not occur simultaneously at both the site near base 29 and the site near base 70. We infer that ribosome interaction at one of the sites may be transient or sterically hindered. Consistent with this view, fragments 69–91

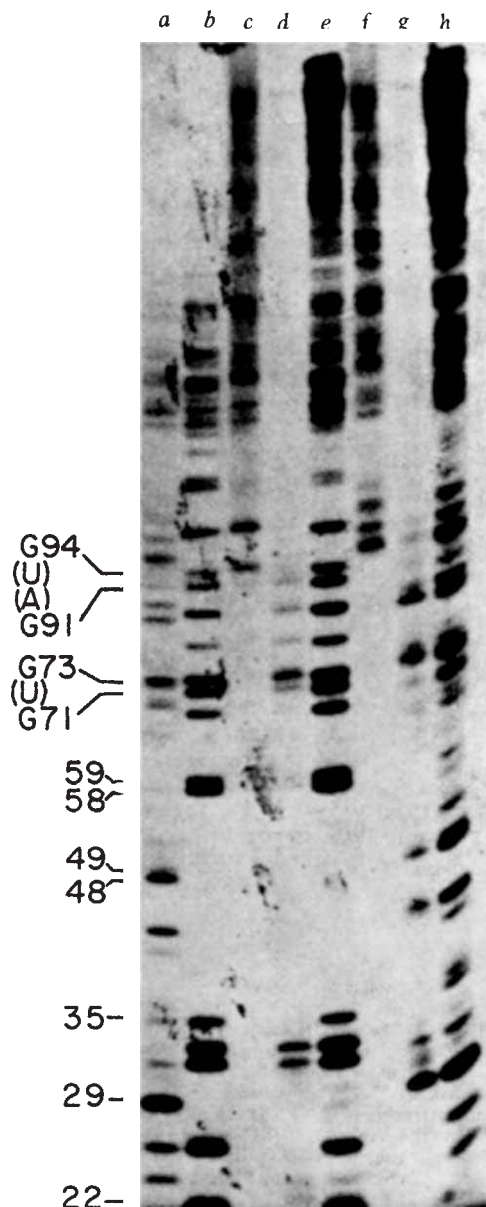


Fig. 4 Analysis of 5' terminal BMV RNA3 fragments bound in monosomes and disomes. Terminal labelling, ribosome binding, and sucrose density gradient sedimentation were as described in Fig. 1. RNA bound to ribosomes was recovered from sucrose density gradients by pooling the desired fractions, adding 120 μ g of wheat embryo ribosomes to pooled disome fractions, and ethanol precipitating the ribosome-mRNA complexes. Precipitates were suspended in 0.1 ml of 0.1 M Tris-HCl pH 7.6, 0.2% SDS, and extracted with 0.2 ml of water-saturated phenol. RNA was ethanol precipitated from the aqueous phase. Partial RNase digestions were performed as described in Fig. 2, except that urea was omitted from digests with RNase ϕ 1, and were phenol extracted once and ether extracted twice before the RNA fragments were ethanol precipitated. The following samples of 5' 32 P-labelled BMV RNA3, each in 10 μ l of 8 M urea, 0.05% xylene cyanol, bromophenol blue were co-electrophoresed at 25 mA on an 8% acrylamide, 7 M urea slab gel (20 $\times$ 40 $\times$ 0.2 cm) in 87.5 mM Tris-borate pH 8.3, 2.75 mM EDTA: *a*, One half of the RNA3 recovered as full-length molecules from the disome peak shown in Fig. 1c, digested subsequently with RNase ϕ 1. *b*, One half of the RNA3 recovered as full-length molecules from the disome peak shown in Fig. 1c, digested subsequently with RNase T1. *c*, RNase T1 fragments recovered from the disome peak shown in Fig. 1d. *d*, RNase T1 fragments recovered from the monosome peak shown in Fig. 1d. *e*, An aliquot of the RNase T1 digest used for the gradient shown in Fig. 1d, saved before binding to ribosomes. *f*, RNase ϕ 1 fragments recovered from the disome peak of a gradient analogous to that shown in Fig. 1d. *g*, RNase ϕ 1 fragments recovered from the monosome peak of the gradient discussed in *f*. *h*, An aliquot of the RNase ϕ 1 digest used for the gradient discussed in *f*, saved before binding to ribosomes. Long exposures of the gel shown reveal the presence of bands corresponding to fragments 69–91 residues long in lanes *c* and *f*.

bases long can form disomes, but only inefficiently, confirming that one of the two sites is poor at binding ribosomes stably.

The method above, by examining an entire set of overlapping fragments simultaneously, permits detailed analysis of the contribution of the mRNA sequence to ribosome binding. By use of suitable end-labelling techniques, this method should be generally applicable to other mRNAs, both capped and noncapped. However, it must be recognised that the nucleotide sequences of fragments may fold differently from their counterparts in intact RNA and thus their binding may be different. We have tried to evaluate the steric effect of secondary structure by systematically examining possible folding by computer. We think that it is unlikely to have affected the transition from monosome formation to disome formation between fragments 91 and 94 residues long. The onset of unfavourable secondary structure may explain however why certain 5'-terminal fragments fail to bind stably in monosomes. For example, while fragments 29, 32 and 33 residues long bind well as monosomes, the fragment 35 residues long binds poorly. The 3' end of the 35 residue long fragment can form a small base-paired stem which is at the threshold of stability (Fig. 5a). Fragments longer than

AUG codon. 80S complex formation in the absence of an AUG codon has been described before with a capped synthetic copolymer of A and U (ref. 26), but not to our knowledge with natural mRNA 5' ends. These observations clearly conflict with the proposal of Kozak and Shatkin²⁷ that 60S ribosomal subunits bind to mRNA only after the 40S subunit is bound to an AUG codon. As discussed below, the possibility thus arises that initiator AUG recognition may be accomplished in normal translational initiation by the 80S ribosome rather than the 40S subunit alone.

Disome binding and two-step 80S ribosome binding

Movement of ribosomes between different sites on RNA3 is not directly tested by our experiments. However, if ribosome interaction with the site(s) 5' to the AUG has functional significance in protein synthesis it must be followed by transfer of the ribosome to the AUG site where translation commences. Disome formation with RNA3 thus indicates the existence of a ribosome entry site or alternatively a ribosome holding site. The

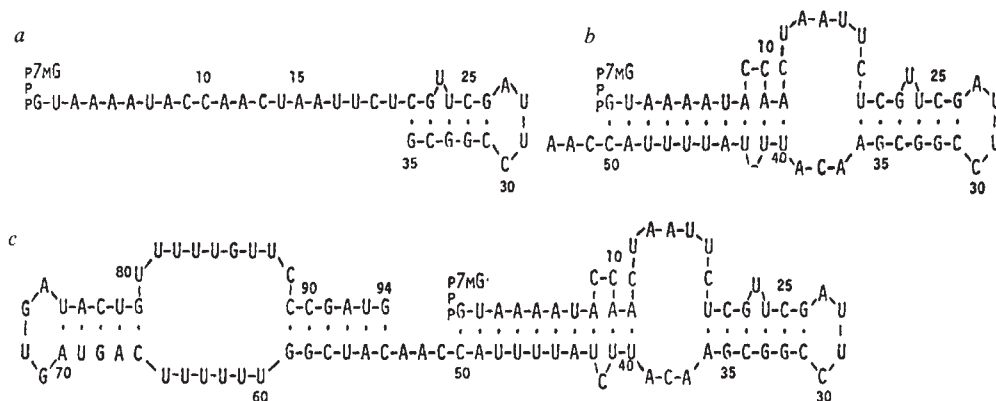


Fig. 5 Possible secondary structures for: a, the first 35 residues of BMV RNA3; b, the first 53 residues of BMV RNA3; c, the first 94 residues of BMV RNA3.

49 and shorter than 72 residues also bind poorly in monosomes and this may be related to stable base-pairing of the extreme 5' end of the RNA which becomes possible (Fig. 5b) in fragments of 50 residues or longer. Alternate base-pairing schemes of theoretical stability comparable to that shown in Fig. 5b can be written for the first 50 bases of BMV RNA3, and transitions between these various structures may also play a part in determining the ribosome binding efficiency of different fragments. As stable base pairing is not possible in fragments shorter than 35 or so residues, though, secondary structure effects have not influenced the transition to competence for monosome formation which occurs between fragments 26 and 29 residues long.

80S monosome formation in the absence of an AUG

Identification of the monosome peaks in Fig. 1 as complexes of mRNA with one 80S ribosome, rather than with two 40S ribosomes, is based on two observations. First, the monosome peak in each gradient coincides exactly with the peak of UV absorbance due to 80S ribosomes (Fig. 1a), unlike complexes of mRNA with two 40S subunits, which are believed to sediment at the 70S position²⁴. Second, complexes of mRNA with single 40S subunits, which would be expected as precursors of complexes of mRNA with two 40S subunits, are completely absent (Fig. 1d). The report²⁵ that a single bound 40S subunit can protect 54 5'-terminal bases plus the cap structure of a reovirus mRNA from nuclease digestion also argues against two 40S ribosomes being able to bind the shortest RNA fragments (29 residues) recovered from the monosome peak. The monosome binding data thus make it clear that capped RNA fragments can bind wheat embryo 80S ribosomes even if they do not contain an

entry site would consist of the cap plus a set of bases involved in the first stable attachment of 80S ribosomes to the RNA. The hypothetical holding site would consist of a set of bases (excluding the cap) that would accept ribosomes from an entry site and release them to a protein synthesis initiation site.

Why is there a sudden jump in efficiency of disome formation at 94 residues? Ribosome protection experiments as well as weak disome formation with fragments as short as 69 residues suggest that there is sufficient room to accommodate two ribosomes on fragments shorter than 94 residues. High efficiency disome formation therefore seems to be specifically related to recognition of the AUG codon at bases 92-94. Possibly the reason disome formation is suppressed in fragments lacking an AUG is that some feature near the 5' end of the RNA involved in binding the second ribosome remains attached to or covered by the first ribosome until the latter is stably bound at an AUG codon.

Our observations indicate that the binding of eukaryotic ribosomes to mRNA is (at least) a two step process. We suggest that eukaryotic 80S ribosomes first bind to mRNA at an entry site 5' to the initiation codon. After this binding the ribosome moves to an initiation site, recognised primarily by the presence of an initiation codon, where translation will begin. An intervening transfer to a ribosome holding site may also occur. Our observations are consistent with the possibility that 80S ribosomes move from the entry/holding site to the AUG by sliding along the mRNA, as suggested by Kozak and Shatkin for 40S ribosomal subunits²⁷. Alternatively ribosomes may be brought close to the AUG by folding of the mRNA. Figure 5c shows a relatively weak, though stable, folding of the first 94 residues of BMV RNA3 in which the cap region and the initiation codon are adjacent to each other. After ribosome binding at the entry site,

melting of the secondary structure and reorientation of the ribosome around the AUG-containing portion of the message could give a stable complex such as those revealed by nuclease protection experiments. In cases where the 5' untranslated region of the mRNA is long enough to permit the entry and initiation sites to be physically separated, such as BMV RNA3 and TMV RNA²⁸, movement of the first bound 80S ribosome from the entry site to the initiation site allows a second ribosome to bind at the entry site. In other mRNAs, particularly those such as BMV RNA4 with a short 5' untranslated region, the entry and initiation sites may overlap or coincide, allowing only a single 80S ribosome to bind when translational elongation is

blocked. A fused entry and initiation site may effect a high efficiency of initiation as is the case with BMV RNA4⁷, while physical separation of the entry and initiation sites might allow a more sophisticated regulation of translation.

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Infrared observations of SS433

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SS433 has attracted much attention following the interpretation of its optical spectrum by Margon *et al.*¹ and Liebert *et al.*². A possible explanation of the Doppler-shifted Balmer emission lines has been proposed by Rees and Fabian³ and Abell and Margon⁴ in terms of two opposed jets that are ejected from the central source at speeds approaching 0.25c and may extend for ~50 AU (~7 × 10¹⁴ cm). Previous work has shown SS433 to be variable at radio wavelengths⁵, X rays⁶, IR^{7,8} and also in the visible^{9,10}. We report here simultaneous observations at IR and visual wavelengths which show that SS433 has an IR excess. We attribute this to a region of gas that radiates by free-free and free-bound emission processes with a total emission measure of the order of 10⁶¹ cm⁻³.

We observed SS433 in bands BVJHK on three nights during the week 14–21 May 1979, from the Cabezon Observatory, Tenerife, using the 1.5 m IR flux collector. Our observations were made using the Leicester University IR/visual photometer with a standard set of filters and an InSb detector operated at 63 K. The standard used was θ Aql, the magnitudes being taken from Johnson¹¹. The reduced fluxes are shown in Table 1

together with the phases (ϕ) computed from the ephemeris of Martin *et al.*¹².

These results show three pronounced features: first, an enormous apparent excess of IR over optical flux; second, an almost flat spectrum for wavelengths >1.2 μ m; third, a variation of IR and optical flux together on a time scale of several days. The first feature is certainly exaggerated by the heavy reddening of the object and may even be entirely due to it, so we have plotted in Fig. 1 corrected fluxes, assuming a value for the interstellar extinction of $A_v = 6$, and a standard extinction curve¹³. The errors on each point are too small to represent on the figure. Taking $A_v = 6$ corresponds to Clark and Murdin's¹⁴ estimated colour excess of $E(B - V) = 2$.

The correction for reddening leaves clear evidence for an intrinsic IR excess; for $A_v = 6$ the IR points all lie above the extrapolation of the optical continuum, whose slope also agrees well with the spectrophotometry of Margon *et al.*¹, corrected for $A_v = 6$.

This conclusion is further supported by Glass' (ref. 7) observation that $K - L = 0.71$. With $A_v < 6$ the IR continuum is even flatter. Since it is generally assumed (see ref. 1) that $A_v \geq 5$ we shall consider $A_v = 6$ as a representative case; it will be clear how the following remarks are affected by assuming other values of A_v . In particular, for large assumed values of A_v , the optical continuum is always much steeper than the IR continuum.

In interpreting these data we must explain the clear intrinsic excess at wavelengths $\geq 1 \mu$ m; four possibilities are investigated here.

(1) High-frequency continuation of the radio spectrum: SS433 is a variable (~factor 3) radio source^{15,16} with a nonthermal spectral index varying between -0.6 and -0.48. With the flux levels quoted in the GHz range, even the most

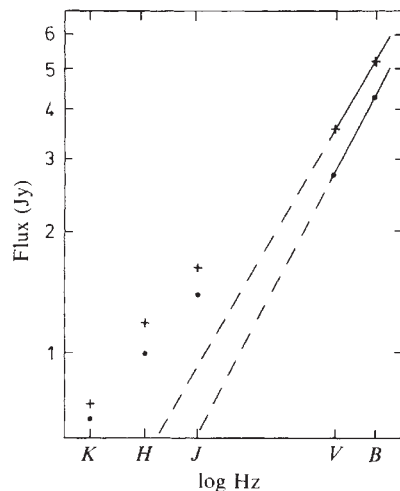


Fig. 1 Corrected fluxes from SS433 assuming that the interstellar extinction $A_v = 6$. Measurements taken on 19 (•) and 21 (+) May 1979.

optimistic extrapolation gives a flux ≤ 1.5 mJy at $1 \mu\text{m}$, as opposed to the observed ~ 500 mJy. Hence some way of causing the nonthermal radio spectrum to flatten above 10 GHz must be found if this hypothesis is to be sustained; this does not seem to be the simplest explanation in view of possibilities (3) and (4) below.

(2) Circumstellar dust: this can be discounted almost immediately since to cause spectral flattening near $1 \mu\text{m}$ the dust would have to radiate at black body temperatures $\geq 4,250$ K, at which temperatures it could not survive. Also the absence of strong forbidden line radiation^{1,2} implies that the gas mixed in with any dust must be fairly dense ($\geq 10^8 \text{ cm}^{-3}$) and would tend to destroy the dust by sputtering.

(3) A red stellar component: a black-body continuum at a temperature near 4,250 K will account for the spectral flattening near $1 \mu\text{m}$ as noted in (2) above. If this is due to a star its spectral type must be near K. Taking $A_v = 6$ and attributing the K magnitude of the object entirely to this hypothetical star we find $M_K \approx -5.4$ at the usually assumed distance of 3.5 kpc (ref. 1). A colour $V - K \approx 2.7$ appropriate to a K-type star gives $M_v \approx -2.7$ at 3.5 kpc, so a star near spectral type K3II is indicated. Since M_v for the system is less than -4.5 with $A_v = 6$ the red star's spectrum would be completely unobservable in the optical. This explanation clearly requires the system to contain at least two components; a binary hypothesis is attractive if accretion on to a compact object turns out to be the underlying 'engine' for the relativistic gas motions. The main difficulty with this explanation of the IR data is the observed variation of the IR flux; apart from the steady increase reported here, Glass⁷ observed variability of 0.8 mag at $2.2 \mu\text{m}$. A steady variation might conceivably be due to long-period binary motion, but any more complicated variability⁸ would probably have to be attributed to some other component of the system. If the variability is substantial this other component must supply most of the IR flux, so that an explanation of the spectral flattening would still be lacking.

(4) Free-free and free-bound emission from an optically thin gas: the continuum spectrum of such a gas at temperatures $\sim 10^4$ K would provide a good fit to the observed IR spectrum, allowing for some 'contamination' at short wavelengths due to the intrinsically steep optical continuum (if $A_v \geq 6$). Evidence favouring this hypothesis comes from the great strength of $\text{H}\alpha$; with $A_v = 6$ the reddening near $\text{H}\alpha$ implies an unattenuated flux of order $10^{-9} \text{ erg s}^{-1} \text{ cm}^{-2}$ in the three observed components^{1,2}. If $\text{H}\alpha$ is interpreted as a recombination line, as seems reasonable, this implies that the plasma emitting it produces a free-free continuum of order 500 mJy at frequencies $\nu \ll kT/h$, where T is the electron temperature of the plasma. (The ratio of the total strength of recombination $\text{H}\alpha$ to the free-free continuum at such frequencies is almost independent of temperature). In

Table 1 Reduced fluxes observed from SS433* and phases

	14	May 1979 19	21
<i>K</i>	414 ± 38	420 ± 16	453 ± 13
<i>H</i>	420 ± 31	435 ± 16	518 ± 19
<i>J</i>	298 ± 65	364 ± 14	426 ± 24
<i>V</i>	11.9 ± 2.2	11.0 ± 0.6	14.1 ± 0.6
<i>B</i>	—	2.73 ± 0.40	3.38 ± 0.25
$\phi^\dagger$	0.138	0.169	0.181

* Fluxes are given in millijanskys with errors of $\pm 1\sigma$.

† Phases computed after Martin *et al.*

other words, if $\text{H}\alpha$ is produced by recombination, the continuum emission from the gas producing it will give an IR continuum of just the observed order if $A_v \approx 6$. (Thus hypothesis (3) above would require also that $\text{H}\alpha$ be produced from a predominantly neutral gas to explain the lack of observed free-free emission, unless A_v is assumed ≤ 4). If the IR flux ≈ 500 mJy is interpreted as free-free emission from a gas near 10^4 K an emission measure $N_e^2 V$ of order 10^{61} cm^{-3} is required, with only a weak dependence on the assumed temperature, assuming a distance of 3.5 kpc. The night-to-night variability in the IR implies a typical size $R \leq 10^{15} \text{ cm}$ for the emitting region; with $V \sim R^3$ we see that the average electron density $N_e > 10^8 \text{ cm}^{-3}$, accounting for the absence of observed forbidden-line emission. These limits imply a free-free optical depth $\tau_{\text{ff}} \geq 10^{23} \nu^{-2}$, so the continuum will be optically thick and decline as ν^2 for frequencies $\nu < 10^{11} \text{ Hz}$; hence it will be unobservable in the radio region. To ensure that the continuum is optically thin at observed IR wavelengths implies $R > 10^{12} \text{ cm}$, $N_e < 10^{12} \text{ cm}^{-3}$. The emission measure 10^{61} cm^{-3} would imply a total cooling rate of order $10^{36} \text{ erg s}^{-1}$, much of which (in the absence of forbidden-line cooling) should appear as UV resonance lines. This cooling rate is of the same order as the observed 2–10 keV X-ray luminosity⁶ ($3 \times 10^{35} \text{ erg s}^{-1}$ at 3.5 kpc) and thus suggests photoionisation as the energy input to the emitting gas. As this is likely to lie far out from the central object, possibly near the ends of 'jets'^{3,4}, it would not intercept the line of sight to the X-ray source and hence not lead to an intrinsic photoelectric turnover in the X-ray spectrum. If photoionisation is the energy supply of the gas, the variability in the IR is readily explained by the variability of the central source, as observed in X rays.

We conclude that the IR emission from SS433 is most easily explained by free-free and free-bound emission from a region of gas with $10^{12} < R < 10^{15} \text{ cm}$, $T_e \sim 10^4 \text{ K}$, $10^8 < N_e < 10^{12} \text{ cm}^{-3}$ and a corresponding emission measure of the order of 10^{61} cm^{-3} , although an additional stellar component cannot be ruled out. More detailed speculation clearly requires both long term IR monitoring and some simultaneous IR-optical-X-ray observations.

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Infrared observations of SS433

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The special features of SS433 have been chronicled over the past 12 months¹⁻⁴. Most unusual of its features is the triple set of hydrogen and helium emission lines. Whilst one of these sets remains at rest, the other two exhibit velocity peregrinations of amplitude 0.27 *c*. These moving satellites have been interpreted^{5,6} as outflowing gas in directly opposing jets which precess with a period of 164 days. I describe here observations of SS433 in the 1–4- μ m spectral region made in March and April 1979 with the 3.9-m Anglo-Australian Telescope. These observations show that the Paschen and Brackett lines of hydrogen also exhibit moving satellites, substantiate the high reddening deduced from optical data, and indicate surprisingly strong free-free emission in the near IR.

All observations were made in daylight using the newly constructed IR photometer-spectrometer. Spectra covering the 2.0–2.5- μ m region at a resolution ($\lambda/\Delta\lambda$) of 50 were taken on seven occasions from March 9 to April 15. All show emission features due to hydrogen. Within this spectral region only $B\gamma$ appears at zero redshift, at a wavelength of 2.17 μ m. The moving satellite lines of $P\alpha$, $B\beta$ and $B\delta$ have also been identified, with the help of the ephemeris of Abell and Margon⁵. Table 1 lists numerical data on the spectra. Three of the spectra are shown in Fig. 1, and the motion of the satellite lines is evident from a comparison of the March 11 and April 6 data. In addition, the prominence of the satellite lines varies on a daily time scale, as can be seen from the March 9 and 11 data, and Table 1. Similar intensity fluctuations of the satellite lines are evident in much of the data (mostly unpublished) of various observers using the Anglo-Australian Telescope.

It would be of interest to compare the intensities of $B\gamma$ and the Balmer lines, and hence to derive a direct estimate of the

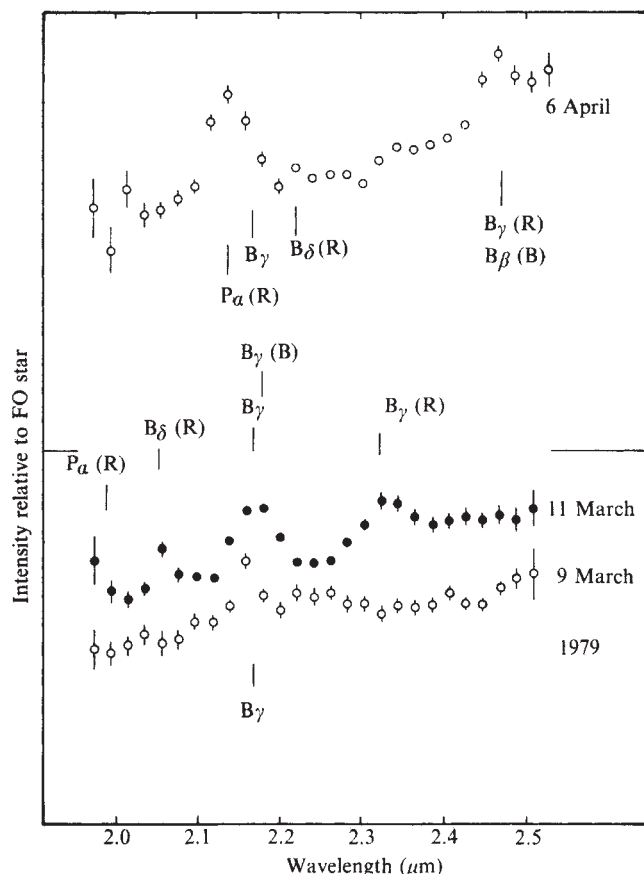


Fig. 1 SS433 2- μ m spectra on three dates in 1979. Each spectrum was divided by that of the FO star ξ Ser to remove the telluric features, and all are reproduced at the same scale. The emission lines marked are the rest wavelength of Brackett γ , and the expected positions of the moving satellites. The latter are coded R and B according to whether the optical equivalents normally appear to the red or blue of rest.

Table 1 Observed emission lines in SS433 and their identifications

UT date 1979	Observed wavelength (μ m)	Identification	Predicted wavelength (μ m)	Equivalent width ($\text{\AA}$)
9 March	2.16	$B\gamma$	2.17	130
	no satellite lines present			
11 March	2.06	$B\delta(R)$	2.07	40
	2.17	$B\gamma$	2.17	130
		$B\gamma(B)$	2.18	
	2.33	$B\gamma(R)$	2.31	100
6 April	2.14	$P\alpha(R)$	2.14	200
		$B\gamma$	2.17	
	2.22	$B\delta(R)$	2.22	10
	2.47	$B\beta(B)$	2.46	120
		$B\gamma(R)$	2.47	
9 April	2.15	$P\alpha(R)$	2.15	230
		$B\gamma$	2.17	
	2.41	$B\beta(B)$	2.43	100
	2.48	$B\gamma(R)$	2.49	—
10 April	2.15	$P\alpha(R)$	2.16	240
		$B\gamma$	2.17	
	2.41	$B\beta(B)$	2.42	80
	2.48	$B\gamma(R)$	2.49	70
13 April	2.16	$B\gamma$	2.17	200
	no satellite lines present			
15 April	2.17	$B\gamma$	2.17	240
		$P\alpha(R)$	2.18	
	2.39	$B\beta(B)$	2.39	100

reddening. So far, this comparison has not been possible due to the lack of concurrent optical data. The relative intensities of the rest and moving features appear to be much the same at visible and IR wavelengths, suggesting that all three gaseous regions experience similar reddening. The equivalent width of the $B\gamma$ line, which can be observed uncontaminated by satellite lines only when the latter are absent, on two of the seven spectra is $170 \pm 30 \text{ \AA}$; this figure may be overestimated by the inclusion of the strongest of the satellite lines ($P\alpha$) on the second occasion.

Photometric observations were possible on only five occasions, due to the presence of cirrus at other times, and only on March 9 were data taken at all four available wavelengths of 1.25 (*J*), 1.65 (*H*), 2.2 (*K*) and 3.8 μ m (*L'*). Over the month of observation the star monotonically brightened by half a magnitude at *J*, *H* and *K*. Of this increase, 0.3 mag occurred between 9 and 11 March (Fig. 1). The IR brightening has since halted according to the 1979 May data of Glass⁷, and an abrupt fading by 0.2 mag between 14 and 15 April was reported by Milone⁸.

It is necessary to correct the measured broad-band data for the influence of the emission lines. On 9 March the satellite lines were weak, and a reasonably accurate correction can be determined. $B\gamma$ contributes about 0.03 mag to the *K* datum. Using a reddening estimate of $A_V \sim 5$ (see below) and case B hydrogen line recombination ratios^{9,10} for temperatures of the order of 10^4 K , the other corrections were estimated as follows: to *J* 0.03 mag due to $P\beta$ at 1.28 μ m, to *L'* 0.04 mag due to $B\alpha$ at 4.05 μ m. The corrected continuum magnitudes are: *J* = 9.76 ± 0.04 ; *H* = 9.10 ± 0.02 ; *K* = 8.45 ± 0.02 ; *L'* = 7.63 ± 0.05 .

Two distinct estimates of the reddening experienced by SS433 have been attempted. One makes assumptions about the under-

lying star, which although plausible are not conclusive for such a controversial object. The other relies on a proved correlation between the reddening and the depths of certain unidentified absorption bands in the visible spectra of reddened stars. These estimates agree on a value for $A_V \sim 6-7$ (refs 1, 3). A third estimate can be derived from the IR photometry. This also makes assumptions about the underlying star, but the assumptions are perhaps more plausible than those pertaining to the optical region alone. In addition, the very presence of $B\gamma$ when He , which shares the same upper level, is too faint to have been observed indicates substantial reddening.

No mechanism is known at near IR wavelengths that produces a spectrum steeper than the Rayleigh-Jeans tail of a black body. For the observed $J-H$ colour, this imposes an upper limit of $A_V \leq 8$. Correction for $A_V = 8$ does not give a match to a Rayleigh-Jeans tail, in the sense that there is excess radiation at longer wavelengths. This we should in any case expect, due to free-free emission from the gas which produces the emission lines. A satisfactory fit can be achieved by a combination of a hot star and free-free emission for $4 \leq A_V \leq 6$. This also reproduces the observed (variable) visual magnitude of 13-14 (refs 3, 11) for K in the observed range 8.0-8.5. For $A_V = 5 \pm 1$, the contribution of the free-free component near $B\gamma$ can be estimated as $60 \pm 10\%$, and thus the equivalent width of $B\gamma$ on March 9 in terms of the free-free continuum is about $210 \pm 40 \text{ \AA}$.

For gas temperatures in the range 5,000-20,000 K the expected equivalent width of $B\gamma$ relative to the free-free continuum^{10,12} ranges from 900 to 400 $\text{\AA}$. A value in this range

was measured by the same equipment in the planetary nebula NGC 2440. An equivalent width of 210 $\text{\AA}$ implies an excessively high temperature; a better hypothesis is that an additional free-free continuum is present. One source of such a continuum is the gas producing the satellite lines. However, as the only measurements of the $B\gamma$ equivalent width were made when the satellite lines were weak, any free-free emission from the gas producing them would also be expected to be weak. Another possibility is that an abnormally high abundance of elements heavier than helium is present in one or all of the gaseous systems. The possible association of SS433 with a supernova remnant^{1,2} lends some credence to this suggestion. Finally, a very small amount of dust radiating at a colour temperature of 1,000-1,500 K could provide the extra continuum required.

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H2155-304: a highly luminous BL Lac object

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The radio source PKS2155-304 has recently been identified¹ with a HEAO 1 X-ray source and a ~ 14 mag object that has a featureless spectrum and is interpreted as a member of the BL Lac class. We describe here our optical spectroscopy of this object which reveals two weak emission features at $\lambda 5,820$ and $5,870 \text{ \AA}$. We suggest that these lines are [O III] $\lambda 4,959, 5,007$ redshifted by $z = 0.17$. This result supports the interpretation of H2155-304 as a highly luminous BL Lac object.

The discovery of an intense soft X-ray source, H2155-304, at high galactic latitude² using the HEAO 1 A-2 experiment was followed by an accurate location with the A-3 modulation collimator³. This enabled a ~ 14 mag object to be proposed as the optical counterpart¹ which is within 1 arcs of the flat spectrum, point radio source PKS2155-304 (refs 4, 5). Optical spectroscopy of the candidate star^{1,6,7} has shown that: (1) it is blue, $B - V < 0.1$; (2) it has a featureless spectrum, with upper limits to emission lines of 2-4 $\text{\AA}$ (ref. 7) and $\sim 1 \text{ \AA}$ (ref. 1); and (3) it is variable in magnitude and colour on a time scale of months. Polarisation measurements¹ also showed a significant ($\sim 5\%$) wavelength dependent polarisation which decreased over ~ 3 months while the object brightened. The object was also found to be associated with a faint, asymmetric nebosity¹. This evidence is all highly suggestive of an elliptical galaxy whose nucleus contains a BL Lac object. Spectral features in BL Lac objects are extremely weak (if detectable at all)⁸ as is evidenced by the difficulty in observing the emission lines from

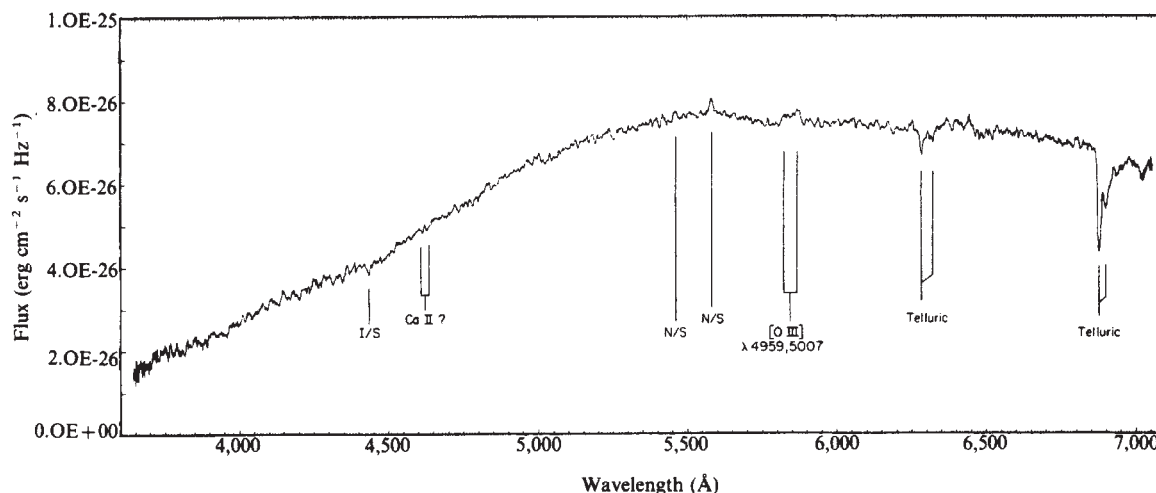


Fig. 1 Lick 3-m ITS spectrum of H2155-304 summed from three nights of observations in October 1978. The marked feature at $\sim 5,850$ is identified with [O III] $\lambda 4,959, 5,007$ redshifted by $z = 0.17$. Features marked N/S are due to improperly subtracted night sky lines; the broad absorption bands are due to atmospheric O_2 .

Table 1 Comparison of properties of X-ray emitting BL Lac objects

Source	<i>B</i>	Ref.	<i>F</i> (mJy)	<i>F</i> ν (GHz)	Ref.	$L_x(10^{46} \text{ erg s}^{-1})$ 2–10 keV	Ref.	<i>d</i> (Mpc)	Ref.
Mkn 421	11.6–16.5	21	570	2.7	15	0.01–0.5	3	180	18
Mkn 501	13.5–14.5	16,17	1,450	2.7	15	0.016	3	200	18
PKS0548–322	14.9–16.1	16,20	310	2.7	4	0.06	3	410	19
2A1219+305	15.7–16.9	3	54	1.4	14	—	—	?	—
PKS2155–304	12.8–14.2	1	280	1.4	5	1.7	1	1,020	This paper

the nucleus of BL Lac itself⁹ and the surrounding galaxy¹⁰. The optical spectroscopy of H2155–304 reported so far has either been of very poor spectral resolution ($\sim 40 \text{ \AA}$)⁷ or of limited sensitivity due to short observation time^{1,6}. We therefore decided to undertake a series of observations of this object with the Lick Observatory 3-m Shane reflector and image tube scanner (ITS)¹¹ to acquire spectrophotometric data of high sensitivity. Our observations were made during the period 24–27 October 1978 and were reduced to an absolute flux level¹² by comparison with observations of standard stars¹³. Several integrations taken in good seeing conditions through relatively large ($4 \times 4 \text{ arc s}$) observing apertures, and guided so as to minimise the effects of atmospheric dispersion, yielded a mean *V* of 14.2 ± 0.1 and *B*–*V* = 0.5 ± 0.2 , substantially redder and fainter than the object appeared 6 weeks later¹. The total mean spectrum of H2155–304 is shown in Fig. 1.

Of principal interest in this spectrum is the somewhat broad, double-peaked emission feature centred on $\lambda 5,850$. Night sky features (due to imprecise sky subtraction) are marked N/S. If we interpret the emission feature as arising from two lines at $\lambda 5,820$ and $\lambda 5,870$ we derive equivalent widths of 0.54 and $0.96 \text{ \AA}$, weak, but certainly significant at this signal-to-noise ratio. Additional support for the reality of these lines comes from: (1) there are no night sky lines that, if improperly subtracted, could give rise to such a feature; (2) the lines are present on all three nights that this object was observed although at a reduced level of significance so that the feature is only statistically significant in the summed spectrum; (3) irregularities in the image tube would be extremely unlikely to produce such a feature because we adjusted the grating setting so as to cover a different wavelength range each night; thereby any real feature would move along the tube (as observed) whereas a tube 'hot spot' would not.

Comparison with the BL Lac spectrum⁹ immediately suggests the identification of the H2155–304 lines with [O III] $\lambda 4,959$ and $\lambda 5,007$. The implied redshift is $z = 0.17$ and hence $L_{\text{opt}} = 7.5 \times 10^{45} \text{ erg s}^{-1}$. At this redshift we have marked in Fig. 1 the implied position of Ca II H and K which might be present weakly in absorption (although there are other stronger features than this which are unidentified). Typically, these lines are easily resolved in active galactic nuclei with this instrument; in our spectrum, they are only marginally separated. An alternative identification for our feature, which circumvents this difficulty, is Doppler-broadened H β emission. However, the higher Balmer lines are not observed, rendering this hypothesis less attractive, unless the Balmer decrement is quite steep.

At this redshift H2155–304 is ~ 50 times brighter than BL Lac itself and in Table 1 the optical, radio and X-ray properties of the four definitely-identified X-ray emitting BL Lac objects are compared with H2155–304. The $\sim 10 \text{ arc s}$ nebula associated with H2155–304 has a physical diameter $\sim 50 \text{ kpc}$ at the Hubble distance of 1,020 Mpc (assuming $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$) which, it has been pointed out¹, is not inconsistent with a large elliptical galaxy. It would therefore be extremely valuable to use the technique of Miller *et al.*¹⁰ to search for absorption lines in the galaxy spectrum and thereby (1) verify the redshift and BL Lac designation, and (2) investigate the stellar contribution to the nebula and compare it with those around QSOs. Given the variability of this object, its continued study spectroscopically is of considerable importance in the search for other lines and variability in the [O III] feature.

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A relationship between the amplitude of the susceptibility and the frequency of the maximum dielectric loss

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We recently presented a new theory of dielectric behaviour based on a many-body cooperative model of interacting dipoles¹. It was shown that the experimentally observed non-Debye behaviour of many dielectric materials² can be represented by these interactions, and a relationship for the frequency dependence of the susceptibility which describes the observed behaviour was presented. The temperature dependence of the simplest dielectric materials which exhibit a dipole alignment transition has now been examined in detail³. One consequence of the cooperative theory is particularly novel and is reported here.

In a conventional dielectric the amplitude of the susceptibility is taken as proportional to the inverse temperature, or as proportional to the inverse of the difference between the temperature and the Curie temperature in a ferroelectric material. The frequency at which the loss peak occurs is simply activated, with the activation energy representing the potential difference between the meta-stable dipole potential minima and the maximum in the barrier between them⁴. It is known, however, that in the region of a phase transition, or the Curie temperature, that the frequency of the loss peak tends asymptotically to zero at the critical temperature⁵. The many-body

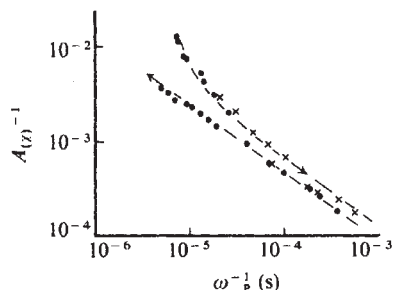


Fig. 1 A plot of the inverse of the amplitude of the susceptibility against the inverse of the frequency of maximum loss for $\text{AgNa}(\text{NO}_2)_2$ from the measurements of Gesi. Data from ref. 7 (O) and ref. 8 (x). The gradient of the parallel lines is -0.71 .

theory^{1,3} gives both of these features as a direct consequence of the basic assumptions. Furthermore the model predicts a set of intrinsic relationships between the magnitude of the susceptibility, $A_{(x)}$, and the peak frequency, ω_p . We have reinterpreted published data to show that one of these conditions has in fact been observed experimentally.

From equation (17) of ref. 1 the frequency at which the peak in the dielectric loss occurs is given by

$$\omega_p = \nu_e \cosh \left(\frac{B + kT_c M_e}{kT} \right) \{1 - (1 - M_e^2)T_c/T\} \quad (1)$$

The significance of the symbolism is given in that reference. From equations (27) and (31) of ref. 1 the magnitude of the susceptibility is proportional to

$$\omega_p^n \cos \left(\frac{n\pi}{2} \right) \zeta^{-n} M'_{(0)} \times F(\omega/\omega_p)$$

where $F(\omega/\omega_p)$ is the shape function normalised to ω_p . On substituting for the zero time value of the mean magnitude of the z component of the local unit dipole vector perturbation, $M'_{(0)}$, this expression can be written in the form

$$A_{(x)} = \left(\frac{\omega_p}{\zeta} \right)^n \cos \left(\frac{n\pi}{2} \right) \frac{(1 - M_e^2)^2}{kT} \{1 - (1 - M_e^2)T_c/T\}^{-1} \quad (2)$$

In the region of the critical temperature, T_c , the terms in parentheses in equations (1) and (2) dominate both the peak frequency and the amplitude of the susceptibility. Under this condition it follows that

$$A_{(x)} \propto \omega_p^{-(1-n)} \quad (3)$$

Alternatively if the temperature T is not close to T_c the activated term in equation (1), ν_e , dominates and

$$A_{(x)} \propto \omega_p^n \quad (4)$$

These relationships show the generality of the parameter n (ref. 6), which has been shown to be the measure of the degree of correlation of individual tunnelling dipolar transitions in the medium¹.

The properties of $\text{AgNa}(\text{NO}_2)_2$, which is a ferroelectric crystal with a low transition temperature (37.8°C) have been reported by Gesi^{7,8}. A compilation of these measurements is shown in Fig. 1, in which the inverse of the susceptibility amplitude has been plotted against the inverse of the peak frequency. The inverse functions have been used as the tracing technique for susceptibility normalisation described in ref. 2 results in temperature shift points which are these inverse functions. Figure 2 shows two parallel lines, one for temperature less than the critical value and the other for temperatures above T_c . The lowest temperatures investigated show a deviation away from the linearity condition towards the region of positive gradient contained in equation (4). The gradients of the lines are $-0.71 = -(1 - n)$ and comparable to the equivalent value of $-(1 - n)$ measured from the high frequency region of the normalised susceptibility/frequency plot for this material of 0.68. Similar results

have been observed from Nakamura and Hosoya's measurements⁹ on $\text{Ca}_2\text{Sr}(\text{C}_2\text{H}_5\text{CO}_2)_6$. In this case the magnitude of $(1 - n)$ was 0.99.

We know of no alternative explanation for this specific inter-relationship between the amplitude and the frequency of the maximum in the dielectric loss. Hence Fig. 1 presents direct experimental evidence for the validity of the many-body, cooperative, model proposed earlier.

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Stable ridges in a collapsing monolayer

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The nature of monolayer collapse has long interested physical chemists studying surface phenomena¹⁻³, and is also of interest to biologists seeking cell-membrane models⁴, since monolayers of fatty-acid type molecules are often used as models for each half of the biomembrane bilayers, and double layers or bilayers may well form on monolayer collapse. Furthermore thin-film properties of fatty acids are similar in many ways to those of cholesterol, lecithins, and other important components of biomembranes. There is little direct experimental evidence to support proposed collapse mechanisms, however. In this note a remarkable type of monolayer collapse is described. Pressure-area isotherms for 2-hydroxytetracosanoic (cerebro) acid rise steeply and bend over sharply at a high pressure with the subsequent falloff in pressure expected for such rigid ulms. Electron micrographs of the collapsing film clearly show many folds or ridges of unusual stability. The compressed monolayer thus provides a model for membranes and thicker films under compression.

2-Hydroxytetracosanoic acid, obtained in high purity from Supelco Inc., was spread from a dilute benzene solution onto distilled water of pH 7.0. Pressure-area measurements were made using the Langmuir-Adam-Harkins technique^{1,5}. Film samples were transferred to Formvar-coated electron microscope grids by a modified Langmuir-Blodgett method⁶. Transferred films were shadowcast in the direction of compression with platinum-palladium at an angle of 10° before examination in a Siemens Elmiskop 101 (ref. 7).

The data for duplicate determinations of the pressure-area isotherm are plotted in Fig. 1 and demonstrate the high reproducibility of the measurements. Extrapolation of the linear portion of the isotherm gives a cross-sectional area of $26 \text{ \AA}^2$ per molecule. Monolayer compressibility is $0.0047 \text{ cm dyn}^{-1}$ and the collapse pressure (without falloff) is 68 dyn cm^{-1} . Lycopodium tests demonstrate the extreme rigidity of the film. Compression-expansion-recompression experiments in the low-pressure region show negligible hysteresis. Water insolubility and surface mobility are thus established. [If any appreciable quantity of benzene is retained in the surface in our conditions it has little effect on the significant part of the pressure-area isotherm (the upper linear portion). These upper linear portions virtually coincide for benzene, hexane, and no

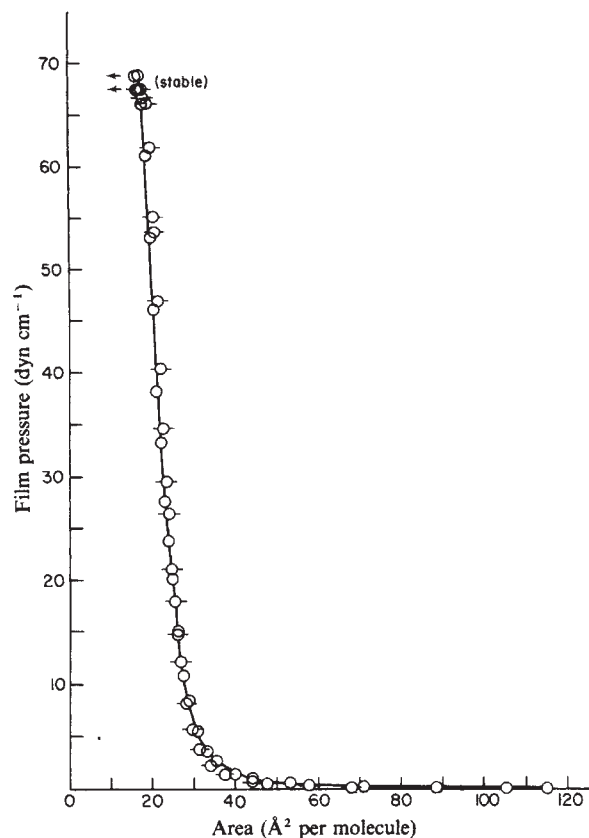


Fig. 1 Pressure-area isotherms for 1-hydroxytetracosanoic acid at 22 °C

solvent^{8,9}. In contrast ethanol has a major effect on the isotherm¹⁰.]

Essentially all rigid or solid-like films have previously shown a precipitous fall in pressure at the collapse or maximum pressure. A probable explanation is that such films break up into double-layer fragments as shown schematically in Fig. 2. Earlier electron micrographs of transferred films have supported this mechanism in which long narrow double-layer platelets form in four successive steps: weakening, folding, bending and breaking^{3,11}.

In Fig. 3, micrographs of the collapsing film of 2-hydroxytetracosanoic acid show essentially no broken fragments. Most striking is the high concentration of tall ridges or folds and the absence of fallen ridges or flat platelets. The ridges are oriented normal to the direction of compression. Many are approximately 2,000 Å in height or the equivalent of about 400 horizontally oriented molecules. In previous studies of solid-type films, such as those of fatty acids and cholesterol, only a few relatively isolated ridges were observed before the appearance of broken double-layer platelets^{3,5,11}. The breaking off of these platelets from the monolayer proper probably accounts for the

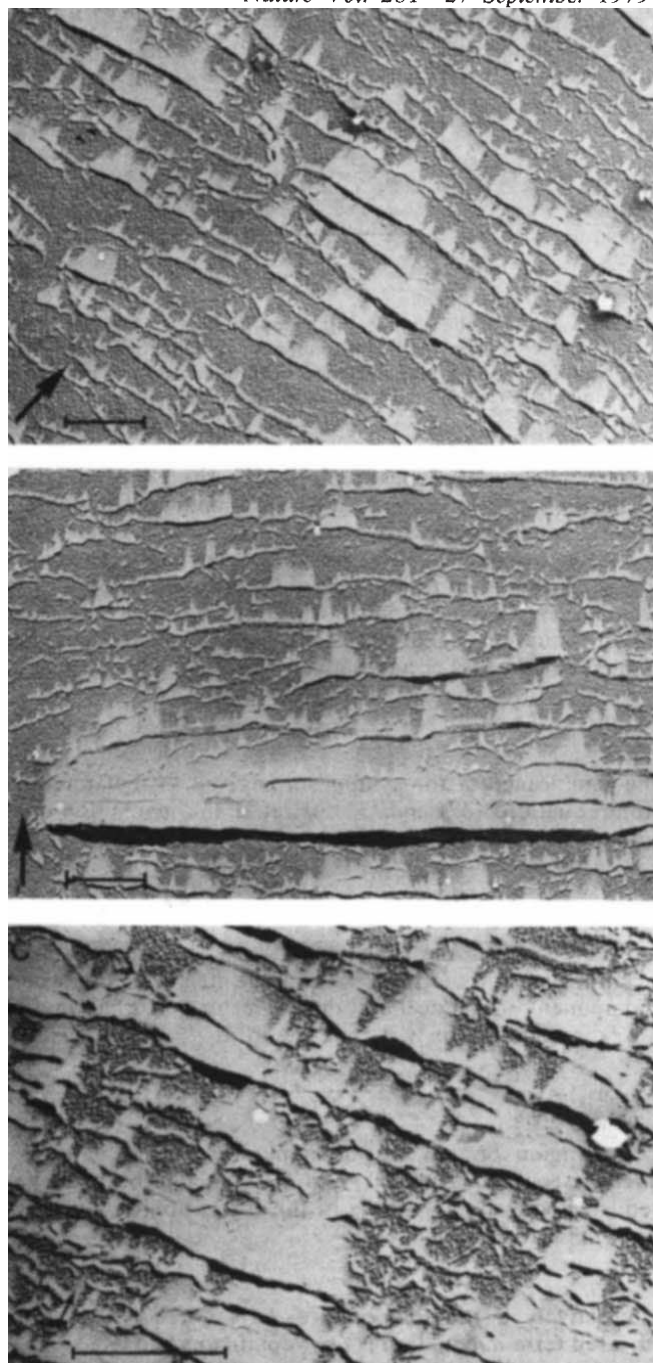


Fig. 3 Electron micrographs of collapsing films of 2-hydroxytetracosanoic acid. Arrows indicate the direction of shadowcasting and shadows appear as light areas. Scale bars, 1 μm.

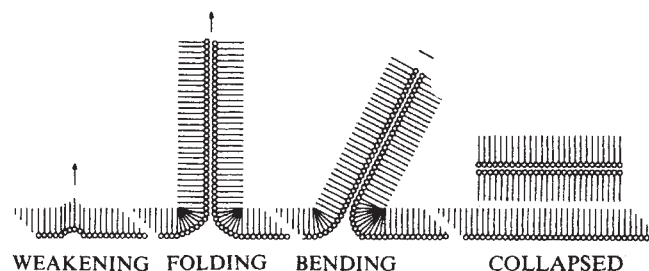


Fig. 2 Mechanism of monolayer collapse.

precipitous fall in pressure usually observed. Without the breaking off of the ridges in the case of 2-hydroxytetracosanoic acid, the monolayer continues to exert its maximum pressure.

Evidently the ridges have great stability in that they withstand the mechanical and thermal shocks of transfer, degassing and shadowcasting. Stability of the ridges and the remaining monolayer may well be provided by the cohesion of the 24-carbon atom chains combined with the intermolecular adhesion (H-bonding) of the hydroxy and carboxy groups. Furthermore, the ridges may provide a reservoir to replenish molecules lost from the monolayer. Collapsed or broken-off platelets would not provide a readily available reservoir because the polar groups of their component molecules could not easily contact the water surface.

Both the concentration and orientation of the ridges provide strong evidence that the compressed monolayer behaves much as a thicker sheet. The monolayer of 2-hydroxytetracosanoic acid may thus serve as an important model for cell membranes and other thicker film systems.

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Total gas content and surface elevation of polar ice sheets

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Interpretation of stable isotope profiles from polar ice cores in terms of past climate requires a knowledge of the elevation at which the ice found at any depth was formed. Measurement of the total gas content of polar ice, V , can give such information if the volume of the pores in ice is known for the time they close off from the atmosphere at the ice formation site¹. For the 1,387-m long core from Camp Century in north-west Greenland, estimates, based on the assumption of a constant pore volume at close-off, suggested that, on the average, ice from the Wisconsin/Weichsel period was formed at an elevation about 1,200–1,400 m higher than that from the Holocene². This would account for an important part of the large shift in the stable oxygen isotopic ratio in this core between late Wisconsin and Holocene ice that was observed by Dansgaard and others³. However, if the pore volume at close-off depends on the site of origin for the ice, estimates of elevation of origin by the gas content method can be seriously in error unless the effect can be allowed for quantitatively. We present here results that enable us to describe the variations in the total gas content of ice that has been formed in present-day conditions in Antarctica and Greenland for a wide range of temperature (–12 to –53 °C) and elevation (400 to 3,200 m). Using these results, the gas content method can now be applied with much greater confidence than before to indicate past elevation changes.

The total amount of gas measured in ice formed by dry sintering of snow can be expressed as:

$$V = V_c \frac{P_c}{T} \frac{T}{P} \quad (1)$$

in which V is reduced to standard temperature T (K), standard pressure P , and 1 g of ice; V_c (per gramme of ice) is the pore volume when the pores are finally closed off in the firn at temperature T_c (K) and air pressure P_c . The conditions required for applying equation (1) are discussed in ref. 4. P_c and T_c depend mainly on the atmospheric pressure and temperature

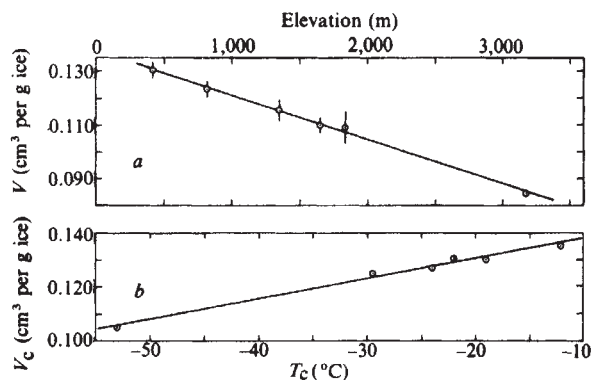


Fig. 1 Total gas content of Antarctic and Greenland ice versus the elevation of formation site *a*, and derived pore volume at close-off versus temperature at the formation site *b*. Standard deviations of the measured mean V values are indicated in *a*.

prevailing at the snow surface and, in the case of recently formed ice, they can be estimated from meteorological surface observations and temperature measurements in the drilled holes.

In order to analyse how the total gas content depends on present-day conditions, we have performed 142 determinations of V on ice recently formed in Antarctica and Greenland and recovered by drilling at six different locations (Table 1). The gas was extracted by melting the ice and refreezing the meltwater under vacuum, then dried and collected in a volumetric gas burette using a Toepler pump. Each measurement of V was performed on 20–25 g of ice, representing a thickness of 2.5–3.5 cm in the glacier. The relative precision of the measurements has been analysed from repetitive determinations of V on natural ice sampled from the same 2.5–3.5 cm thick layers. About 50 sets of such repetitive measurements were performed. The difference between these comparable measurements in V never exceeds 2% and is on the average 0.7%.

There are systematic differences, however, between results obtained using different procedures for extracting the gas under vacuum. For two locations, Camp Century and New Byrd, the current procedure yields mean V values (see Table 1) systematically 0.006 cm³ per g of ice smaller than those previously reported by Raynaud⁴. This does not affect gas content changes in ice deduced from the results of a single procedure, and gas content changes obtained by different procedures may be compared.

Samples have been selected among the pieces of core available to obtain the best value for the amount of gas trapped when firn turns into ice under present-day and dry conditions. In particular, core measurements have been taken from unfractured sections that are apparently undisturbed by percolating meltwater and well below the pore close-off zone (since near this zone the pore seals may be delicate and consequently changes in the original gas content can occur between core recovery and measurement). At each drilling location, ice flow modelling results and isotopic profiles of the ice indicate that the layers investigated here were formed during the Holocene, when climatic conditions were similar to today and at approximately the same site. (At Dome Summit, Camp Century, and Dome C the investigated layers were most probably formed near the drilling site; but in the case of Cape Folger, D 10, and New Byrd the origin of the ice is considered to be different from the drilling location due to glacier flow and, in the case of New Byrd, to changes in ice thickness.)

The number of determinations of V performed for each site was between 5 and 58. Corresponding mean values with standard deviations are shown in Table 1. The variations observed for a given site are generally larger than experimental errors and are, in part, due to short-term natural fluctuations. The mean values of V show a significant and mostly proportional decrease

with respect to the elevation (E) of the ice formation site (Fig. 1a). The least-squares linear regression is:

$$V(\text{cm}^3 \text{ per g of ice}) = -1.66 \times 10^{-5} E(\text{m}) + 0.138 \quad (2)$$

with a correlation coefficient of -0.998 which is significant at a $<1\%$ level (Student's t -test).

Calculation of V_c using equation (1), the mean V values, and estimated values for P_c and T_c (Table 1), indicates that about half of the observed decrease in V with respect to E is linked to the change of the ratio P_c/T_c with elevation and that the remainder is due to variations in V_c . Any systematic change in the porosity at close-off must depend primarily on firnification parameters, such as the accumulation rate and the temperature which, in particular, influences the grain size. Our results indicate no significant correlation between accumulation rate and V_c . On the other hand, a very consistent dependence of V_c on T_c is obtained (Fig. 1b) which can be expressed by a linear regression:

$$V_c(\text{cm}^3 \text{ per g of ice}) = 7.4 \times 10^{-4} T_c(\text{K}) - 0.057 \quad (3)$$

the level of significance of the correlation (coefficient = 0.992) being $<1\%$ (Student's t -test).

A further indication of a correlation between V_c and temperature arises from the short-term variations of V recorded by the two continuous sets of increments analysed from the D 10 and Dome Summit cores (Fig. 2). The amplitudes of these variations cannot be due only to experimental errors and fluctuations in P_c and T_c . They probably reflect natural fluctuations in V_c which contribute in a significant way to the observed V variations. The comparison between changes in V and isotopic composition (δD) in Fig. 2 suggests that ice formed from snow deposited in warmer conditions (high δ values) contains a higher pore volume (high V values) than ice formed from snow deposited in colder conditions (low δ).

The results shown in Fig. 1 lead to the following relation between V , P_c and T_c (obtained from equations (1) and (3)):

$$V(\text{cm}^3 \text{ per g of ice}) = \left[7.4 \times 10^{-4} P_c - 0.057 \frac{P_c}{T_c(\text{K})} \right] \frac{T(\text{K})}{P} \quad (4)$$

Thus the total amount of gas trapped in polar glaciers, when dry firn turns into ice, depends on the atmospheric pressure and temperature prevailing at the formation site. In present-day surface conditions, an elevation increase of $1,000$ m corresponds to a decrease in V of about 0.017 cm^3 per g of ice. This gradient is close to those obtained over much smaller elevation

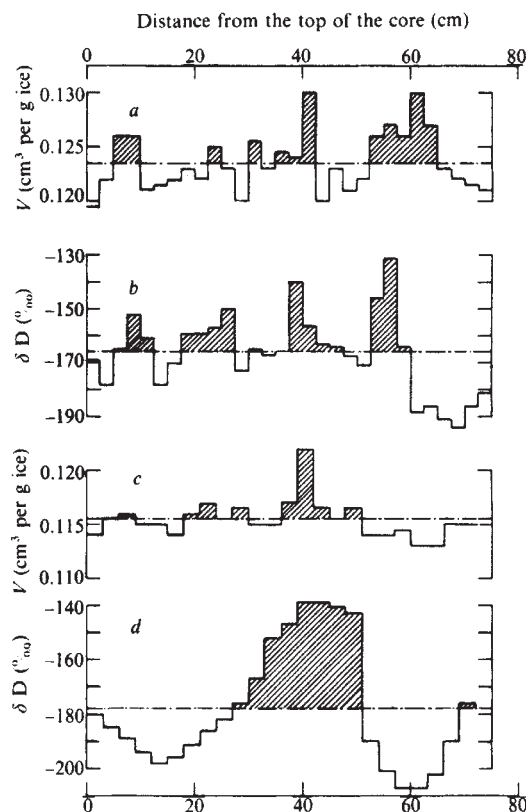


Fig. 2 Total gas and deuterium content variations observed on the two continuous sets of core increments taken at D 10 (depth range: 117.1–117.9 m below the surface), a and b, and at Dome Summit (232.0–232.7 m below the surface), c and d. The dashed lines are the average values.

ranges by Budd and Morgan⁷ for the Law Dome (Antarctica) and from mean total gas content values reported by Miller⁸ for stations Milcent and Crête (Greenland). According to equation (4), a general climatic cooling of 5°C at the ice formation site would imply a decrease in V of approximately 0.001 cm^3 per g of ice. These figures clearly show the potentiality of V as elevation indicator and confirm that the mean difference of

Table 1 Mean total gas content, $\bar{V}$ measured on ice recently formed in Antarctica and Greenland

Drilling site	Depth interval (m below surface)	No. of determinations	$\bar{V} \pm \sigma$ (cm ³ per g of ice)	Elevation* (m)	P_c^* (mbar)	T_c^* (°C)	V_c (cm ³ per g of ice)
Cape Folger, F station (Law Dome, East Antarctica)	131.0–131.2	5	0.1305 ± 0.0025	420	935	−12	0.135
D10 (Terre Adélie, East Antarctica)	117.1–117.9	57	0.1235 ± 0.0030	820	895	−19	0.130
Dome Summit (Law Dome, East Antarctica)	100–233	58	0.1155 ± 0.0030	1,350	825	−22	0.1305
New Byrd (West Antarctica)	100–900	9	0.110 ± 0.003	1,650	795	−29.5	0.125
Camp Century (Northwest Greenland)	200–800	7	0.109 ± 0.006	1,840	795	−24	0.127
Dome C (East Antarctica)	155.0–155.2	6	0.0845 ± 0.0010	3,170	655	−53	0.105

The depth interval at which the ice has been taken as well as estimated elevation, atmospheric pressure (P_c) and temperature (T_c) of the ice formation location are also given for each site. V_c values are the calculated pore volumes at close-off from equation (1).

* From refs 4 and 5, except for New Byrd. For that site, according to ref. 6, the surface elevation at the origin site for the ice found now between 100 and 900 m depth is, on the average, about 150 m higher than the surface of the drill site, and P_c and T_c have been estimated taking into account a pressure–elevation gradient of 0.1 mbar m^{-1} and a temperature–elevation gradient of $0.01^\circ\text{C m}^{-1}$ for the Byrd Station area.

about 0.015 cm^3 of gas per g of ice measured between Holocene and Wisconsin ice in the Camp Century core¹ can be primarily attributed to an elevation effect. Taking into account the present-day V -elevation gradient (from equation (2)), the Wisconsin section of the core is found to be formed, on the average, at an elevation about 900 m higher than the Holocene section (which may be compared with the earlier estimate of about 1,200 m (ref. 2) which was based, among other things, on the assumption of constant V_0). The colder climate during the Wisconsin (compared with the Holocene) leads to a calculated elevation effect somewhat less; thus, if the climatic temperature change is 10°C , the elevation difference would be about 800 m. Considering the present-day rate of decrease in stable isotope composition of ice with elevation of $0.62 \delta^{18}\text{O}\%$ per 100 m for Greenland⁹, our new elevation estimate would account for about $5 \delta^{18}\text{O}\%$ or almost half of the mean isotopic difference between the Wisconsin and Holocene ice at Camp Century.

The gas content method, if extended to a wide range of polar ice cores, can provide unique information on ice origin and past thickness changes of polar ice sheets and thus enables more reliable interpretation of past climatic changes deduced from isotopic measurements on ice cores.

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Denitrification in the nitrogen budget of a river ecosystem

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Recent studies indicate that bacterial denitrification in anaerobic sediments may play a major part in removing nitrogen from water during river transport^{1,2}. As this reaction reduces nitrate-N primarily to gaseous nitrogen, it represents a potentially significant pathway for the permanent removal of nitrogen from aquatic environments. However, no detailed assessments have been made of the quantitative importance of denitrification in relation to river nitrogen budgets. I have investigated this problem by studying the role of denitrification in relation to the nitrogen budget of Duffin Creek near Toronto, Ontario, and report here that the removal of nitrate-N by denitrification in the downstream reaches of the river represents 5–6% of the annual export of total nitrogen from this river basin.

Duffin Creek has an average flow of $2.2 \times 10^5 \text{ m}^3 \text{ d}^{-1}$ and drains a predominantly rural watershed of 262 km^2 . Nitrogen transport was measured at intervals along the two main branches of the river. River discharge measurements were available from recording gauges at sites 1, 2, 3, 6, 8, 11 and 13 (Fig. 1). At the remaining sites current meter measurements coupled with cross-sectional areas were used to calculate discharges at times of river water sampling. Nitrate-N was measured in water samples colorimetrically using an automated cadmium reduction method³. Detailed measurements of all nitrogen forms were also available for sample sites used from government water quality monitoring studies⁴.

During May to October in 1973 to 1975 and in 1978 river discharge and concentrations of nitrate-N were measured on 18 days of low flow discharges. A comparison of river discharges for each channel reach indicated that inputs and outputs of water differed by less than 15% for almost all the observations. The six river reaches examined showed a consistent pattern of daily losses of nitrate-N. The mean daily loss for the 18 days varied between 1.7 kg (40 mg m^{-2} of stream bed) and 20.3 kg (300 mg m^{-2} of stream bed) nitrate-N (Fig. 1). The concentration of ammonium-N and nitrite-N was negligible during low flow conditions. Calculations of organic-N transport revealed no evidence of significant gains or losses.

Uptake of nitrogen by benthic algae and macrophytes seems to account for ~15% of the nitrates removed from the river water (A.R.H., in preparation). The possibility that nitrate losses in Duffin Creek are caused mainly by denitrification in river sediments was investigated in a laboratory experiment. Sediment samples were covered with a solution containing $5 \text{ mg per l NO}_3\text{-N}$ as KNO_3 . Water samples were removed at various time intervals and analysed for nitrate-N. The results indicated a rapid disappearance of nitrate with almost total loss occurring in some samples within 6 days (Fig. 2). Average rates of nitrate-N loss over a 6-day period ranged between 100 and

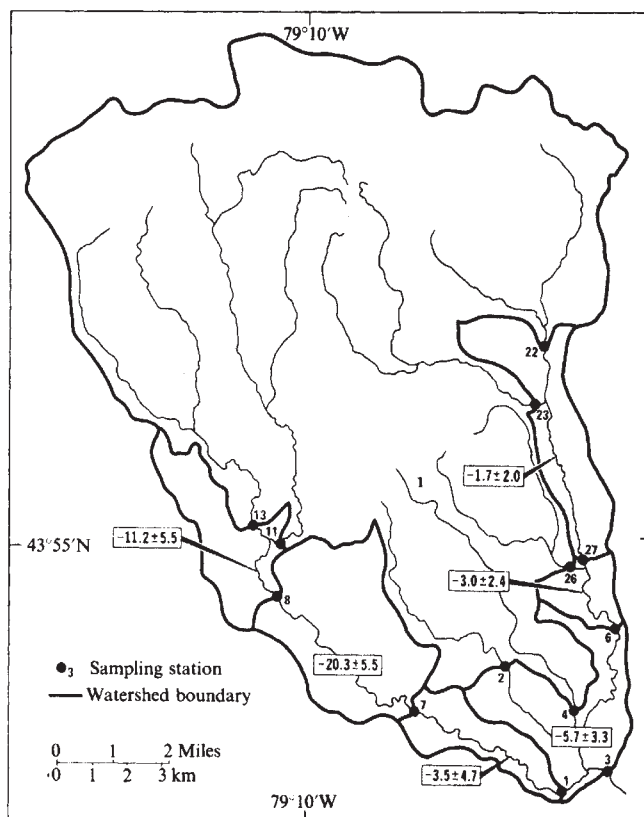


Fig. 1 Mean ($\pm$ s.d.) daily losses of nitrate-N during low river flows in the late May–October period 1973–75 and 1978.

251 mg m⁻² of sediment surface daily for silt rich deposits and between 20 and 60 mg m⁻² for samples with a high sand and gravel content. These rates of nitrate disappearance may provide an overestimate of denitrification losses because assimilatory nitrate reduction by bacteria was not evaluated. However, a recent laboratory investigation of sediments from streams in southern Ontario showed that 90–95% of the nitrate removal resulted from denitrification⁵.

Nitrogen input-output balances were also measured for 10 days during the winter and spring months for the six downstream reaches of Duffin Creek. The data do not show any consistent pattern of nitrate-N removal, but this does not preclude the occurrence of denitrification. Small nitrate losses resulting from low rates of denitrification in cold temperature conditions would probably not be evident in the field data, owing to the range of error involved in the measurement of discharge and water chemistry.

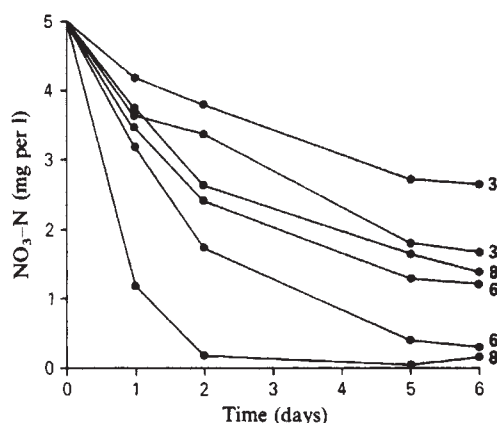


Fig. 2 Changes in nitrate-N concentration in water overlying sediment cores collected from sites 3, 6 and 8. The cores were incubated in the dark at a temperature of 20 °C. Air pumps were used to introduce bubbles close to the water-sediment interface to reproduce the turbulence and aeration characteristic of river conditions.

The disappearance of nitrate-N was observed in 12 sediment-water profiles incubated at temperatures of 20 °C, 10 °C, 6 °C and 1 °C. From December to March the water temperature in Duffin Creek fluctuates between 0 and 2 °C. The laboratory data reveal that denitrification occurs in river sediments even at these low temperatures, although nitrate removal is only ~20% of the rate occurring at summer water temperatures which average 20 °C. River water temperatures range between 4 and 10 °C during November, April and early May. Water-sediment profiles incubated at 6 and 10 °C showed rates of nitrate loss which were 35% and 45% respectively of the nitrate removal at 20 °C. These results suggest that denitrification may occur in Duffin Creek during the winter and spring although at a much lower rate than is characteristic of late May to late October.

The field and laboratory data can be combined to calculate the annual removal of nitrogen by denitrification from the two main branches of Duffin Creek extending from sites 11, 13 and 22 to site 3 (Fig. 1). The 18 sets of field data collected between late May and late October indicate an average daily loss of 45 ± 9 kg (s.d.) of nitrate-N for the combined six downstream river reaches. This daily rate of loss when adjusted to account for uptake by benthic algae and macrophytes would result in the removal of about $6,300 \pm 1,250$ kg of nitrate-N between 20 May and 31 October. Using the laboratory data on rates of denitrification at low water temperatures, it is estimated that an additional 2,000 kg of nitrate-N is lost between November and late May, producing a total annual removal of ~8,300 kg.

This calculation of annual nitrate-N removal ignores the possible effects of high river flows on denitrification. The occurrence of considerable disturbance and scouring of sediments during periods of storm flow probably creates aerated conditions which inhibit denitrification. The relationship between suspended sediment concentration and discharge at site 3 suggests that disturbance of the river bed sediment begins to occur at discharges greater than $2.83 \text{ m}^3 \text{ s}^{-1}$. If it is assumed that denitrification is zero on days which have a mean daily discharge exceeding $2.83 \text{ m}^3 \text{ s}^{-1}$, the annual removal of nitrate-N is reduced to about 7,300 kg.

The average annual export of nitrate plus nitrite-N from the Duffin Creek basin at site 3 for the period from May 1973 to May 1975 was ~73,000 kg (ref. 6). Measurements of Kjeldahl nitrogen concentrations are also available for the same period⁴. Calculations based on these measurements together with the daily river discharge records at site 3 indicate an average annual export of 62,000 kg Kjeldahl-N, giving a total nitrogen loss of 135,000 kg.

These observations allow an assessment of the importance of denitrification in relation to the nitrogen budget of Duffin Creek. The annual removal of 7,300–8,300 kg of nitrate-N in the downstream reaches of the river is ~5–6% of the annual export of total nitrogen from the river basin. This percentage must be regarded as tentative in view of the difficulty in estimating denitrification during the November to May period. Moreover, the budget technique used in this study cannot measure the denitrification of nitrate-N generated within bottom sediments: nitrates produced by nitrification in the oxidised surface layer of river sediment may subsequently undergo denitrification after diffusion to deeper anaerobic zones⁷.

The importance of denitrification in relation to the nitrogen budget of Duffin Creek varies on a seasonal basis. Approximately 80% of the annual nitrogen exports from the river basin occur between November and April, when denitrification is negligible. However, denitrification is a major factor in the nitrogen budget of the creek from late May to October. Low river flows occur on approximately 140 days during these months each year. In these conditions there is an average daily removal of 45 kg nitrate-N in the downstream reaches principally due to denitrification. This loss represents 50% of the average daily input of total nitrogen and 75% of the nitrate-N input received by these channel reaches (A.R.H., in preparation).

The preservation or enhancement of denitrification in rivers has been viewed as a possible approach to the management of nitrogen in the environment^{1,8}. These data suggest that this strategy would probably not produce a major reduction in the annual export of nitrogen from many drainage basins. However, benefits in the form of reduced nitrate-N concentrations in water and smaller nitrate exports to downstream ecosystems could be derived during low flow discharges in the warm seasons of the year.

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Anomalous propagation of seismic waves through the zone of shearing contact between converging plates of the New Hebrides arc

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Results obtained from a network of five short-period seismograph stations operated for 2 months on the island of Santo in the central region of the New Hebrides provide evidence for the anomalous propagation of seismic waves through the zone of shearing contact between the converging plates of the New Hebrides Island Arc.

Santo Island has an abnormal position in the subduction zone, being located where the trench is usually found. The crustal blocks including Santo and Malekula Islands separate the New Hebrides Trench into a northern and a southern segment (see Fig. 1). The evolution of this anomalous morphology, and the relationship of it to the subduction of a major aseismic ridge, the *D'entrecasteaux* Fracture Zone, remain as interesting problems¹⁻⁵. However, recent studies⁶⁻⁹ show that the overall configuration of the Benioff zone is not now significantly contorted or disrupted through the central region of the arc. The inclined zone of thrust faulting or shear contact between the subducted oceanic plate and the island arc appears to be continuous along the entire length of the arc and is located at very shallow depths beneath the island of Santo (Fig. 2).

Thus a network on Santo is in a unique position in relation to the plate boundary, a position attainable in most other subduction zones only by the deployment of ocean bottom seismographs. In particular, a station on the west coast of Santo is seaward of the shallow zone of seismicity in such a position that it receives seismic waves which must pass through the zone of shearing contact between the plates. Stations in eastern Santo receive seismic waves which propagate mainly above the zone of contact, so a comparison of records obtained on Santo can show large anomalies in seismic wave propagation that might be associated with the zone of plate contact.

Five stations were operated on Santo as shown in Fig. 2. Earthquakes were located from arrival times of P and S waves. The accuracy of location is about ± 5 km. The station on the

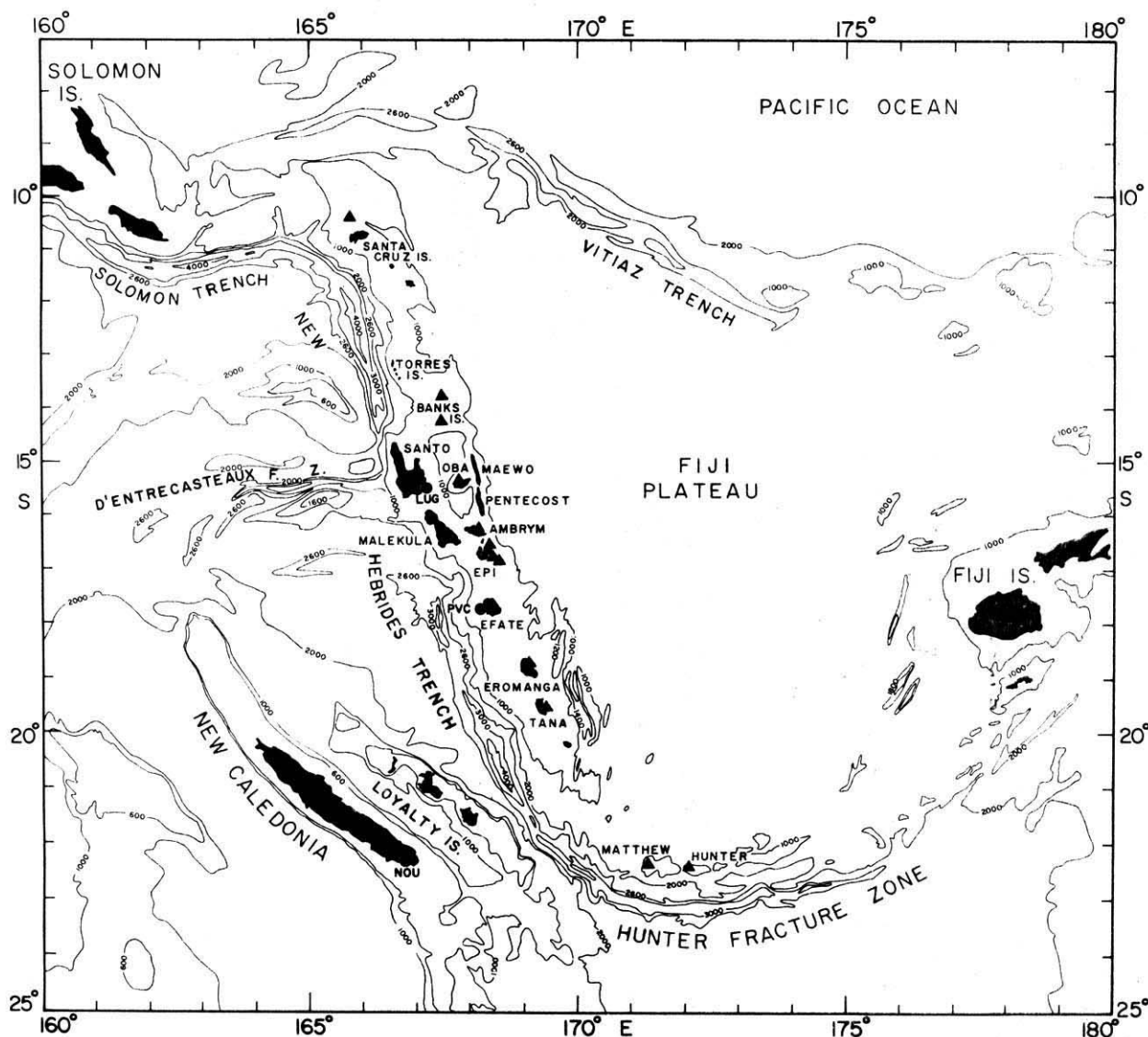


Fig. 1 Map of the New Hebrides island arc in the southwest Pacific region.

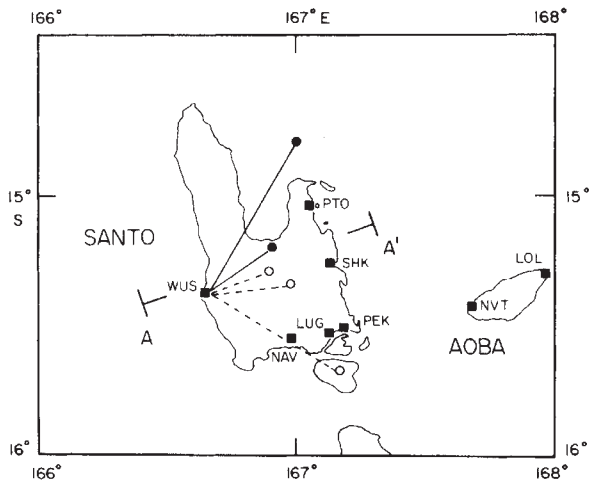


Fig. 2 Map of Santo and Aoba Islands showing temporary seismograph network operated in 1975. The three-letter abbreviations and solid squares indicate the seismograph stations. The solid and open circles show epicentres of the numbered earthquakes shown in Figs 4 and 5. Schematic ray paths to the station WUS are shown from those events. The dashed ray paths indicate low frequency shear waves at WUS.

west coast of Santo, WUS, operated for 10 days. The results for the period when WUS was recording are illustrated in Fig. 3. The dashed line is an approximation of the upper limit of the descending plate (see ref. 9). First motions of P waves from the cluster of events shown at shallow depths in Fig. 3 also indicate that the cluster is within the zone of plate contact. Events such as numbers 26 and 81 (Fig. 4) are reliably located beneath the thrust zone and are within the descending plate.

Although WUS recorded numerous events located beneath the thrust zone at intermediate depths, it clearly recorded only three of the events within the thrust zone (Fig. 3). As shown in Fig. 4, for two of the events (nos 79 and 88) the shear waves recorded by WUS have a frequency content remarkably lower than those from the same earthquakes recorded by stations PEK and SKB located in eastern Santo. The third event (no. 74) produces no clear shear waves at WUS, although the P wave is

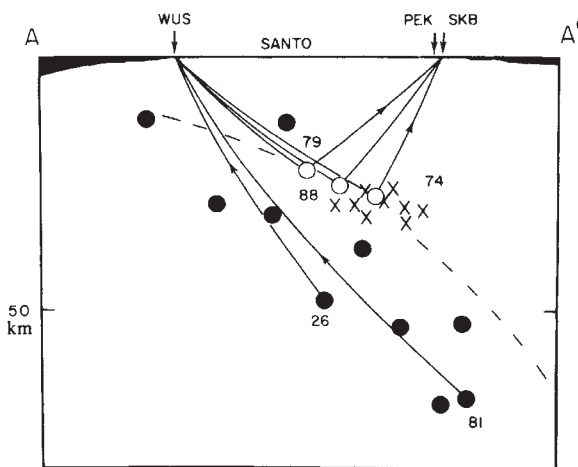


Fig. 3 Vertical section (see Fig. 2) showing events located during the 10-day period during which WUS was in operation. Crosses indicate events not perceptible on the WUS records, and circles indicate events recorded by WUS with (filled circles) or without (unfilled circles) high frequency shear phases. The ray paths are schematic and are not the result of quantitative ray tracing.

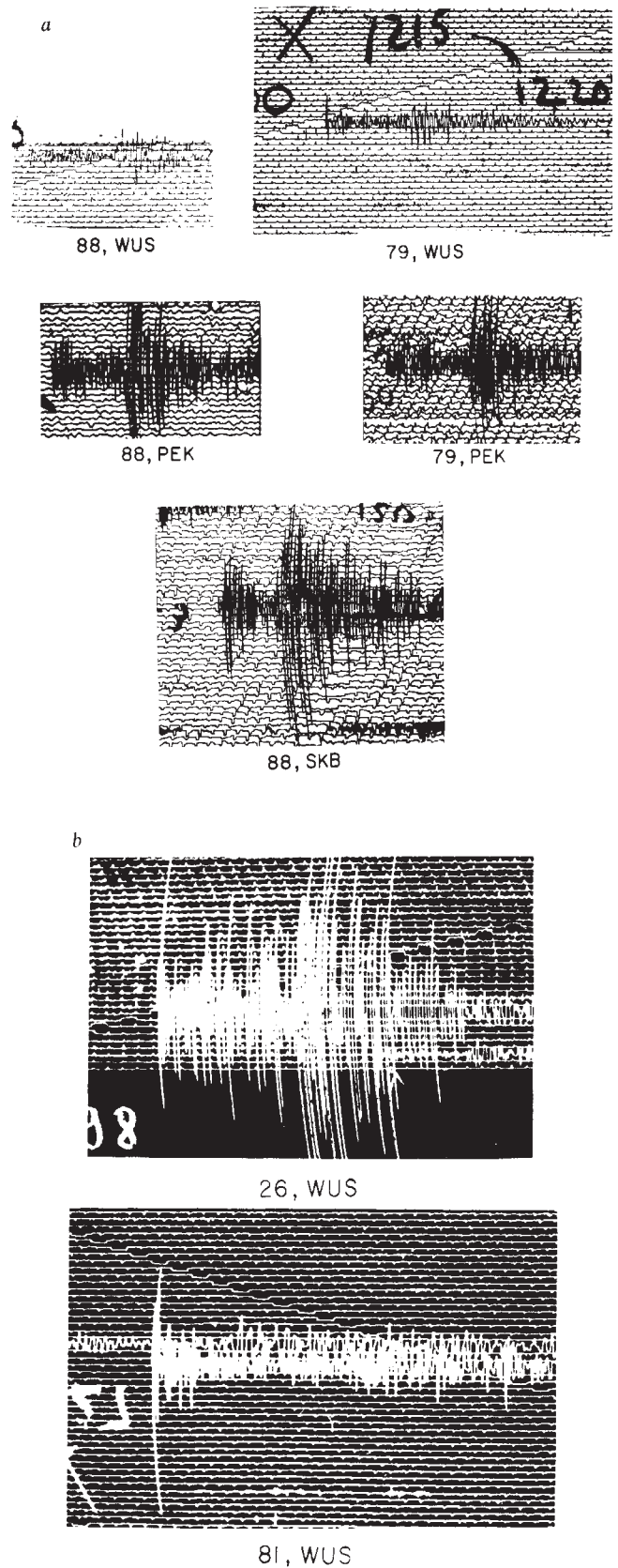


Fig. 4 *a*, Examples of seismograms from stations on Santo. The numbers and stations correspond to those shown in Figs 2 and 3. Second marks are clear on all records; the longer marks in certain records (for example, no. 79, WUS) are minute marks. *b*, Same as *a*, but showing different seismograms.

well recorded there, and high frequency shear waves are well recorded for this event in eastern Santo. In contrast, WUS records high frequency shear waves from events not within the shear zone (for example nos 26 and 81, Fig. 4), as do the other stations on Santo. The low frequency shear waves recorded by WUS for the two events in the thrust zone have predominant frequencies of 2 to 2.5 Hz, whereas the shear waves recorded by WUS for other events and by other Santo stations typically have predominant frequencies of 4 to 10 Hz.

Although the data are few, the combination of paths shown in Fig. 4 indicates that the unusual low frequency shear waves are not likely to be a localised effect near WUS. The anomalies must be a result of either a very special and unusual azimuthal variation in the radiation from the source, or, more likely, an effect of the propagation path. Results obtained so far raise the possibility that the shear waves to WUS suffer anomalously high attenuation within the zone of shearing contact between the converging plates of the subduction zone. The rays from earthquakes within the thrust zone must travel most of the way to WUS through or near the thrust zone. In contrast, rays from other events to WUS or from events within or beneath the thrust zone to other stations on Santo must only cross obliquely or pass above the thrust zone. If the removal of high frequency waves along the paths to WUS is by non-elastic dissipation, it may be possible to relate this to the development of a zone of viscous slip which is thermally activated by shear heating (as discussed by

Yuen *et al.*¹⁰). However, Yuen *et al.* consider that such a zone would probably develop at greater depths and further down along the dip of the plate boundary. Alternatively, the attenuation may reflect scattering associated with heterogeneities developed within a zone of intense shearing. In either case, the zone should have a width sufficiently large to affect the passage of seismic waves.

Clearly further observations must be obtained. However, the data presented here suggest that useful information about the physical properties of a major earthquake-generating plate boundary may be obtained from suitably deployed local seismograph networks.

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A Lower Carboniferous *Lepidodendropsis* flora in Kashmir

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The concept of a more or less uniform world-wide flora (the '*Lepidodendropsis* flora') of early Carboniferous age, was first expounded by Jongmans¹. He cited the fossil plant genera *Lepidodendropsis*, *Triphyllopteris* and *Rhacopteris* as characterising this palaeofloristic unit, of which he claimed representation from a number of Northern and Southern Hemisphere localities. Subsequent work has generally confirmed his conclusion that in the early Carboniferous a floristically rather uniform lycopod-rich association extended into all the major continents, preceding the onset of Carbo-Permian glaciation. In its relative uniformity this flora contrasts sharply with the pronounced regional character of the floras (especially the Gondwana *Glossopteris* flora) that followed the glaciation. Although representation of Jongmans' *Lepidodendropsis* flora was claimed for the major southern land masses (South America, Africa, Australia) only a very limited Carboniferous flora, of a few genera of fern/pteridosperm leaves, has been reported^{2,3} from India. This flora, from the Thabo stage of Spiti, in the Western Himalayas, was the only Indian Carboniferous flora known hitherto, and it differed significantly from its counterparts in other parts of Gondwanaland in the absence of *Lepidodendropsis*, and indeed any other lycopods. A newly discovered flora of Lower Carboniferous age from the Gund Formation of Kashmir, some 400 km west-northwest of Spiti is reported here.

The Gund Formation comprises about 500 m of cyclic sediments consisting of conglomerates, sandstones and shales. It overlies unconformably the Syringothyris Limestone, regarded as of Tournaisian age on faunal grounds, and is overlain by the Fenestella Shale Formation of Upper Viséan to Bashkirian age. It is thus securely bracketed as lying at least within the Lower Carboniferous⁴.

The Gund flora contains several genera which link it with typical *Lepidodendropsis* floras from other parts of the world; it is dominated by a number of lycopods, particularly *Lepidodendropsis* (Fig. 1b), *Lepidosigillaria* (Fig. 1c), *Archaeosigillaria* and *Cyclostigma*, together with *Archaeocalamites* (Fig. 1a), *Rhacopteris* (Fig. 1d, e) and other sphenopterids. In these constituents the Gund flora shows significant agreement with the Early Carboniferous *Lepidodendropsis* flora from Europe, from the Pocono Formation of the US, and floras of comparable age in Peru, Ghana, Morocco, eastern Sahara and Egypt, Syria, China and western Malaysia. The common elements are shown in Table 1.

Jongmans¹ regarded his *Lepidodendropsis* flora as 'the flora of the older part of the Lower Carboniferous and as the transition to the *Archaeopteris*–*Cyclostigma* flora of the true Upper Devonian'. Note that in the Lower Carboniferous of Geigen Hof, Bavaria, where Lutz⁵ first recognised *Lepidodendropsis hirmeri* (the type species), this genus occurs in association with *Rhacopteris* and various other sphenopterids, together with *Sphenophyllum*. Similar, more or less contemporaneous, floras are found in many parts of Europe with minor variations, such as the *Lepidodendropsis*–*Archaeocalamites*–*Rhacopteris* association of North Wales⁶ or the *Lepidodendropsis*–*Archaeocalamites*–*Triphyllopteris*–*Rhacopteris* association of southern Spain⁷. Floras of the Pocono Formation in Pennsylvania and Virginia are strikingly similar to these, and comprise various species of *Lepidodendropsis*, *Cyclostigma*, *Triphyllopteris* and other sphenopterids⁸. A *Lepidodendropsis*–*Adiantites*–*Fryopsis* flora is known as far north as Alaska⁹.

Comparable assemblages occur on the now separate parts of the Gondwanaland mass. A *Lepidodendropsis*–*Cyclostigma*–*Triphyllopteris*–*Adiantites*–*Rhacopteris* assemblage is known from Carhuamayo in Peru¹⁰. In Africa, *Lepidodendropsis* is reported from Ghana¹¹, and from Morocco¹² through Libya¹³ and the eastern Sahara¹⁴ to Egypt and Sinai^{15,16}, in association with one or more of the genera *Prelepidodendron*, *Archaeosigillaria*, *Lepidosigillaria*, *Archaeocalamites*, *Sphenopteris* and *Rhacopteris*. The early Carboniferous age attributed to Australian reports of *Archaeocalamites*, *Lepidodendron*, *Rhacopteris*, *Fryopsis* and *Adiantites* has been questioned¹⁷. In south-east Asia a *Lepidodendropsis*–*Sublepidodendron*–*Archaeocalamites*–

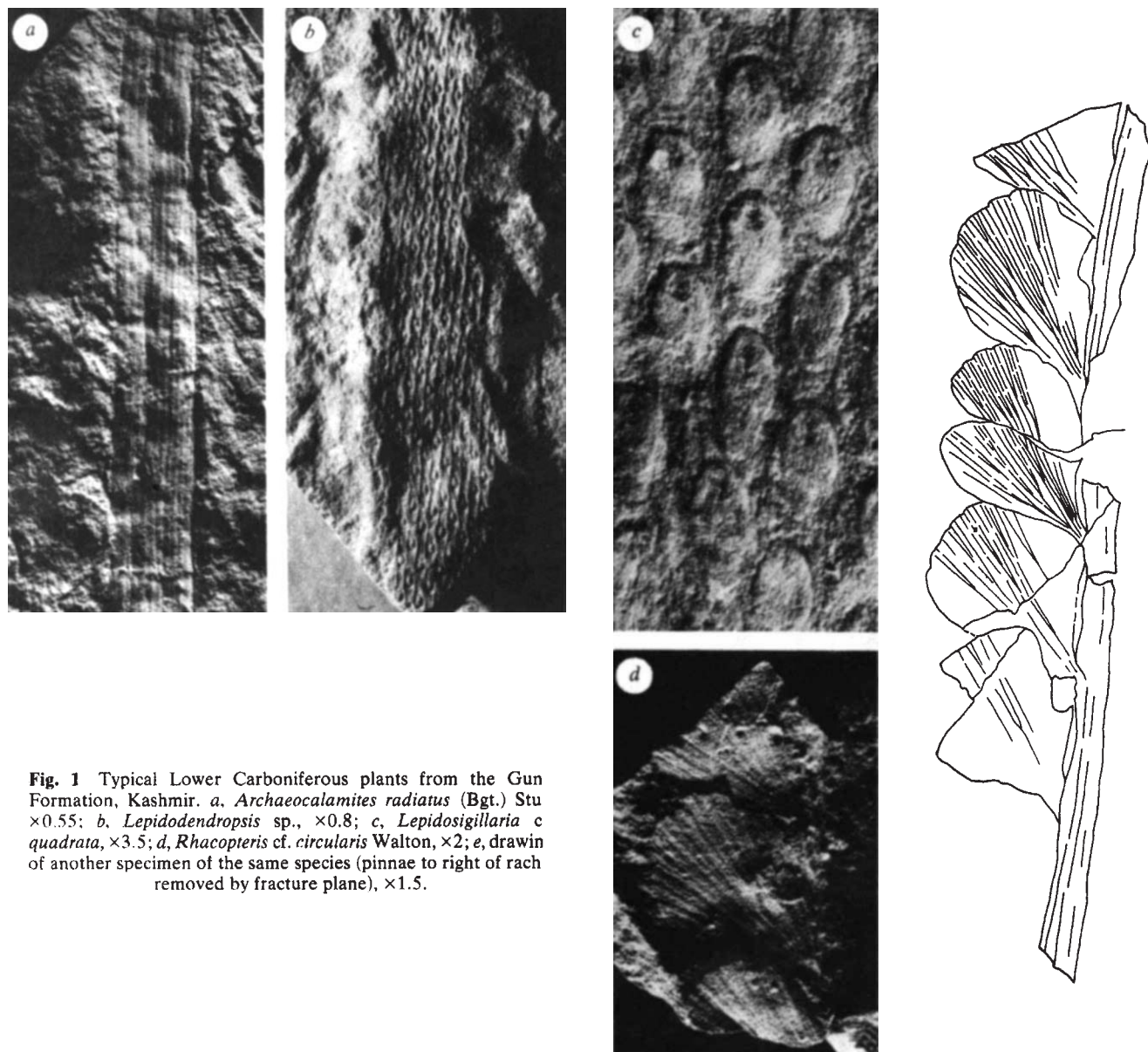


Fig. 1 Typical Lower Carboniferous plants from the Gun Formation, Kashmir. *a*, *Archaeocalamites radiatus* (Bgt.) Stur $\times 0.55$; *b*, *Lepidodendropsis* sp., $\times 0.8$; *c*, *Lepidosigillaria c quadrata*, $\times 3.5$; *d*, *Rhacopteris* cf. *circularis* Walton, $\times 2$; *e*, drawing of another specimen of the same species (pinnae to right of rach removed by fracture plane), $\times 1.5$.

Table 1 Common features of the Gund flora and early Carboniferous floras found elsewhere

Fossil plants in the Gund Formation	Europe (early Carboniferous)	North America (early Carboniferous)	Peru (early Carboniferous)	North & West Africa (late Devonian to early Carboniferous)	China (early Carboniferous)	Malaysia (early Carboniferous)
Lycopsidea						
<i>Archaeosigillaria</i> sp.	*			*		
<i>Lepidosigillaria</i> cf. <i>quadrata</i> Danze-Corsin (Fig. 1c)				**		
<i>Lepidodendropsis</i> sp. (Fig. 1b)	*	*	*	*	*	*
<i>Lepidodendropsis</i> cf. <i>sigillarioides</i> J.G. & D.	*	**	*	*	*	*
<i>Lepidodendropsis</i> cf. <i>fenestrata</i> Jongmans	*	*	*	**	*	*
' <i>Cyclostigma</i> ' <i>ungeri</i> J.G. & D.	*	**	*	**		
Pteropsida						
<i>Rhacopteris</i> cf. <i>circularis</i> Walton (Figs. 1d, e)	**	*	**	*		
<i>Rhodeopteridium tenuis</i> Gothan	**	**			*	*
Sphenopsida						
<i>Archaeocalamites radiatus</i> (Bgt.) Stur (Fig. 1a)	**	**	*	*	**	

** Species in common; * Genus in common.

Triphyllopteris-Cardiopteris association is known from Wutung in Kianshi Province, China^{18,19}. Further south a *Lepidodendropsis-Lepidodendron-Adiantites-Rhodopteridium* assemblage occurs in the Lower Carboniferous of western Malaysia²⁰.

In the area east of the Urals floras occur that are characterised by several endemic genera of lycopods (*Ursodendron, Tomiodendron, Lophiodendron*) together with *Angaropteridium* and *Chacassopteris*²¹. While the separation of these several Russian genera may well be justified, the general aspect of these Lower Carboniferous Angara floras is broadly comparable to contemporaneous floras from other parts of the world⁹.

Almost all known records of *Lepidodendropsis* are Lower Carboniferous in age, apart from two species reported from the Devonian of China²², and the reports by French workers of this and other lycopod genera from the Devonian of North Africa. The reports from North Africa of several genera (*Lepidodendropsis, Archaeosigillaria, Tomiodendron, Abacodendron*) in rocks of Devonian age, considerably pre-dating their first occurrence elsewhere, has led to the suggestion²³ that Africa was the centre of origin of this whole group of arborescent lycopods (*Lepidodendrales sensu lato*). Until the age of the African material can be linked more securely with reliably-dated marine faunas this interpretation must be regarded as controversial.

The age of the Australian *Rhacopteris-Lepidodendropsis-Cyclostigma* flora, originally regarded²⁴ as Lower Carboniferous has more recently been reinterpreted as of Upper Carboniferous (Westphalian) age¹⁷. Such uncertainties in the age of pre-*Glossopteris* floras in Gondwanaland emphasise the significance of a flora such as that of the Gund Formation, in lying within a sequence containing marine faunas above it and below.

The Gund flora reported here confirms the close comparability of the Lower Carboniferous flora of northern India with that of other areas both in Gondwanaland and the (present) Northern Hemisphere. The four well-explored parts of the Gondwana block (India, South America, Africa and Australia) now prove to have had as close a floristic similarity before the Carboniferous glaciation, as that shown by the more intensively studied *Glossopteris* flora which succeeded it. A more detailed comparison of the plants of the Gund flora with those from other parts of Gondwanaland will be presented elsewhere.

Recent palaeomagnetic data from the Silurian and Devonian of China²⁵, has led Keppie²⁶ to divide Eastern Asia into two parts along a line through the Tien Shan-Mongol mountain belts to Vladivostok in his palaeogeographic reconstruction for the Devonian and Early Carboniferous. He was influenced in this proposal by the otherwise puzzling juxtaposition in the same latitudes of the Early Carboniferous Angara floral province with that of *Lepidodendropsis* in China. His reconstruction now places the boundary between these provinces at a palaeolatitude of approximately 35° N.

The present-day Chinese and Malaysian areas are brought in Keppie's reconstruction²⁶ into closer proximity to Gondwanaland than generally visualised hitherto, leaving their *Lepidodendropsis* flora disjunct from that of the main body of Eurameria. A somewhat more westerly position for the Sino-Malaysian block, possibly lying north and west of Australia, would offer a continuity with the *Lepidodendropsis* floras of Africa, South America and Europe, with this newly-discovered flora in Kashmir acting as a link between the eastern and western occurrences of *Lepidodendropsis*.

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A gliding reptile from the Upper Permian of North East England

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In July 1978 an unusual fossil reptile was discovered by D. Hall and myself 1.5 cm above the base of the Upper Permian Marl Slate at Epbleton Quarry near Hetton-le-Hole, Tyne and Wear (NZ 351484). The sapropelic Marl Slate, the first member of the English Zechstein succession, is a marine deposit well known for its fossil fish (mainly palaeoniscids) and land-derived plants¹. The only recorded reptilian remains consist of two incomplete skeletons of *Protorosaurus*². The new find is an incomplete skeleton (the skull, forelimbs and most of the tail have been destroyed by quarrying), 17 cm long, of a terrestrial reptile washed into the Zechstein Sea. It is especially interesting for its elongated and jointed dorsal ribs interpreted as an adaptation for the support of two membranes to enable the reptile either to parachute or more probably glide in a manner similar to the modern gliding lizard *Draco* of South East Asia³.

The reptile is lying on its back and the bones, severely crushed by compaction of the clay matrix, are very fragile. The axial skeleton consists of 36 amphicoelous vertebrae comprising 13 constricted dorsals, 2 sacrals and 21 slightly constricted caudals. On the left side there are 21 elongated ribs, the last six of which articulate with the first six dorsal vertebrae. The remaining 15 ribs converge anteriorly towards the vertebral column until truncated by the edge of the rock slab in which the skeleton is embedded. From this it is evident that at least 15 anterior dorsal vertebrae are missing, and an original pre-sacral count of at least 28 vertebrae is indicated.

All the ribs have a prominent ventral groove and the rib heads are weakly dichococephalous, articulating dorsoventrally with the centra by means of narrow 'shoulders'. Because they lie closely packed together and are partially superimposed, it is difficult to determine the relative lengths of the elongated ribs, although anteriorly they measure at least 130 mm. Several important features are visible on the left side, where each rib is interrupted by a break interpreted as a joint near the rib head (Fig. 1). These joints divide the ribs into a relatively short proximal end (articulating through the rib head with the vertebral column) connected (through the joint) to an extremely attenuated distal end tapered to a hair-like extremity. On the right side this relationship is almost obscured by the attenuated distal rib ends which are tightly folded back against the vertebral column (Fig. 1). The joints probably enabled the distal ends of the ribs, supporting the 'wings', to articulate in a horizontal plane either

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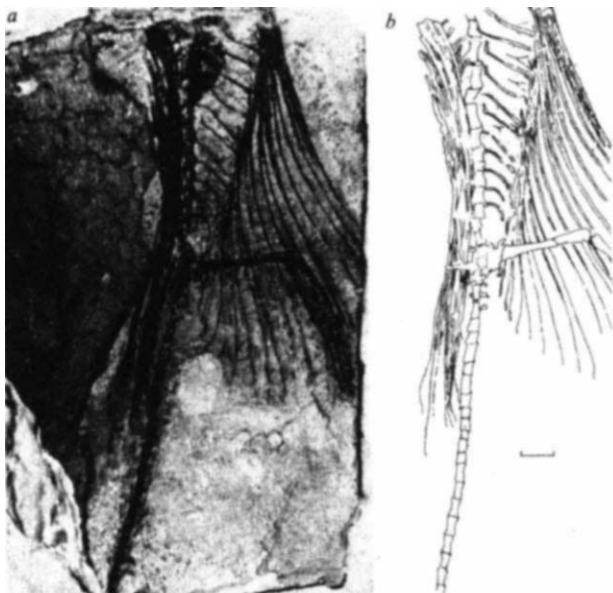


Fig. 1 Photograph (a) and sketch (b) of the reptilian skeleton from the Marl Slate of Hetton le Hole. Scale bar, 1 cm

to spread them for gliding or to fold them back against the body for resting or walking. The thin, sinuous character of these ribs suggests they must have been fairly flexible and it is likely that, as in *Draco*, they were supported and pulled into shape by an elaborate arrangement of muscles and ligaments³. Behind these ribs are four short ribs also characterised by weakly dichopcephalous rib heads.

Although the pelvis is crushed, a posteriorly directed iliac blade is visible. The left hind limb comprises a femur (27 mm long), fibula (18 mm long), tibia (19 mm long) and an elongate, clawed pes of which the tarsus and five digits have been crushed and splintered. The femur, tibia and fibula are relatively slender and thin walled. Of the right hind limb, preserved in counterpart, only fragments of the femur and an almost complete pes are preserved. Four broad-headed metatarsals are each 6 mm long. The fourth digit is 22 mm long with a deep and narrow claw. Several such claws are visible at the extremities of the digits of both right and left pes.

The Upper Permian Kupferschiefer is the European equivalent of the Marl Slate and in Germany has yielded three fragmentary skeletons of gliding reptiles. Only one, the holotype of *Weigeltisaurus jaekeli*, has been fully described^{4,5}, consisting of a well preserved skull with a partially disarticulated skeleton. The classification of *Weigeltisaurus* is uncertain and it has been variously placed in the subclass Lepidosauria order Rhynchocephalia⁴, in the Lepidosauria order Weigeltisauridae⁵ and in the subclass Euryapsida order Araeoscelidia⁶. Although similar to the Hetton reptile, it is larger and differs in the proportions of the skeletal elements. The two other (fragmentary) German specimens, pending a full description, have been tentatively referred to the genus *Weigeltisaurus*⁷ but correspond in size and form more closely to the Hetton specimen. These three small reptiles are probably co-specific but their relationship to *Weigeltisaurus* is uncertain. There are about 15 species of the modern gliding lizard *Draco*, varying in size and weight from 5–6 g to 30 g in *Draco maximus*, the largest species³. By analogy, it is possible that the size range of the Permian gliding reptiles is attributable to different species of the genus *Weigeltisaurus*. Alternatively, they may represent sexual dimorphism within one species. Another gliding reptile, *Daedalosaurus madagascariensis*, has been described from the Upper Permian of Madagascar⁸. This poorly preserved lepidosaurian has 21 pairs of elongated ribs and shows both anatomical differences and similarities to the European reptiles. More research is required to clarify the classification and relationships of all these reptiles.

Similarly adapted reptiles include the Upper Triassic Kuhnosaurs, which possess 10–11 pairs of elongated ribs^{3,9} and species of the aforementioned agamid lizard *Draco*, with 5–7 pairs³. The elongated ribs and other similarities of functional morphology can be related to the specialised ecology of these reptiles exemplified by *Draco*, an insectivorous form living aboreally in the rain forests of South East Asia³. Similar palaeoenvironmental conditions along the Zechstein Sea coasts are indicated by the diverse flora in the Marl Slate^{1,10} and Kupferschiefer¹¹ which has also yielded insects^{12,13}.

I interpret the recurring adaptations in the Permian, Triassic and present-day gliding reptiles as the products of repetitive convergent evolution enabling exploitation of a highly specialised ecological niche.

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Negatively correlated cross-resistance to a synthetic pyrethroid in organophosphorus-resistant *Tetranychus urticae*

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Spider mites are serious pests of food and fibre crops and often cause considerable reductions in yields. Control is usually by regularly spraying chemicals, but frequently this approach has led to the development of resistance to these chemicals and ultimately to reduced levels of control^{1–3}. A change to another chemical is then necessary. Most species of tetranychid mites of agricultural significance can develop resistance to many organophosphorus insecticides, some organochlorine and carbamate insecticides and various other compounds. Cross-resistance, where resistance to one compound confers resistance to other compounds of the same group, and multiple resistance, where resistance has developed to compounds from a number of structurally different groups, are also known to occur. Occasionally the development of resistance to one compound will have the reverse effect by making a population more susceptible to a newly introduced compound. This effect, termed negatively correlated cross-resistance, could have significant practical advantage in restoring some degree of susceptibility in a pest population. In this letter we describe an example of this phenomenon in spider mites in which resistance to organophosphates was linked with increased susceptibility to synthetic pyrethroids.

A strain of two-spotted spider mite, *Tetranychus urticae* Koch, resistant to an organophosphorus (OP) insecticide, was

Table 1 Relative toxicities of azinphos-methyl and fenvalerate to strains of *T. urticae*

Population	Azinphos-methyl			Fenvalerate		
	LC ₅₀ (g l ⁻¹)	95% CI for LC ₅₀	Slope	LC ₅₀ (g l ⁻¹)	95% CI for LC ₅₀	Slope
A	0.564	0.482–0.661	4.71	0.021	0.016–0.028	2.05
B	0.432	0.349–0.533	3.41	0.018	0.015–0.022	4.16
C	0.418	0.340–0.514	3.22	0.019	0.014–0.025	2.43
D	0.408	0.330–0.502	3.15	0.018	0.015–0.036	4.16
E	0.273	0.220–0.340	3.55	0.031	0.023–0.043	2.19
F	0.213	0.170–0.265	2.68	0.022	0.015–0.032	1.93
G	0.108	0.086–0.137	4.82	0.027	0.019–0.040	1.82
H	0.068	0.050–0.093	1.73	0.039	0.031–0.048	3.00
I	0.063	0.034–0.120	2.42	0.041	0.029–0.058	1.97
J	0.058	0.045–0.073	2.43	0.156	0.113–0.214	1.73
K	0.051	0.040–0.065	2.82	0.187	0.015–0.232	3.82
L	0.023	0.018–0.030	2.54	0.079	0.052–0.121	1.53

Toxicity tests were performed by attaching 30 adult female mites to glass microscope slides with double-sided adhesive tape and immersing for 5 s in serially diluted solutions (5–7 concentrations) of the candidate insecticide. Solutions were prepared by dissolving technical grade samples (95%–99% pure) of fenvalerate and azinphos-methyl in an acetone: water (1:1) solvent. To determine solvent-induced mortality, mites were also immersed in solvent alone. Control mortality, which did not exceed 10%, was used to correct concentration-mortality data by Abbott's formula⁸. All dipping was performed at 25 ± 1 °C and 70–80% relative humidity for 24 h.

observed to be more susceptible to a synthetic pyrethroid (SP) insecticide than was a strain which had no history of exposure to insecticides. To confirm this observation populations of two-spotted spider mites from apple orchards in the Canterbury Province, New Zealand, were sampled for further laboratory tests. In these orchards the OP insecticide azinphos-methyl had been sprayed 5–10 times each year for many years to control lepidopterous pests. Spider mites in these orchards have become resistant to this insecticide. As a comparison, spider mite populations from home gardens where no sprays have been applied were also sampled for testing. The toxicities of azinphos-methyl and the SP insecticide fenvalerate were then determined by a standard laboratory slide dip technique⁴. Mortality was assessed after 24 h and the concentration-mortality data were subjected to probit analysis⁵ to determine median lethal concentration (LC₅₀) values. Results for both insecticides are presented in Table 1 and Fig. 1.

Negatively correlated cross-resistance is clearly demonstrated, increasing azinphos-methyl resistance conferring an increase in fenvalerate susceptibility. Populations of two-spotted spider mite with no history of exposure to insecticides were relatively more tolerant of fenvalerate than were field populations subjected to azinphos-methyl selection pressure. This phenomenon is uncommon among spider mites and has only been reported where resistance to the OP insecticide oxydemetonmethyl enhanced susceptibility to the formamidin compound, chlordimeform⁶ and the carbamate insecticide formetanate⁷. Our results therefore suggest that by using fenvalerate a superior level of control could be achieved with

azinphos-methyl resistant populations than with less resistant populations. The inclusion of fenvalerate in spray treatments in orchards where azinphos-methyl resistant mites abound may control these mites and eliminate the need for a specific acaricide. This would conserve the limited resource of effective chemicals.

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A virus which lyses the marine nanoflagellate *Micromonas pusilla*

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Observations of virus-like particles (VLPs) are now commonplace in a wide range of cell types. In most cases these observations represent incidental findings reported during a general ultrastructural investigation. Frequently in such instances it is impossible to establish the viral identity of the observed particles because the sample material containing the inclusions is no longer available and/or the data provided are inadequate. In particular, the number of established viral infections of algae^{1,2} or protozoa³ is small, and those amenable to continued study even smaller. We now report the isolation and ultrastructural identification of a lytic virus infecting the naked, marine phytoflagellate *Micromonas pusilla* (Butcher) Manton et Parke and describe its unusual mode of entry into the host.

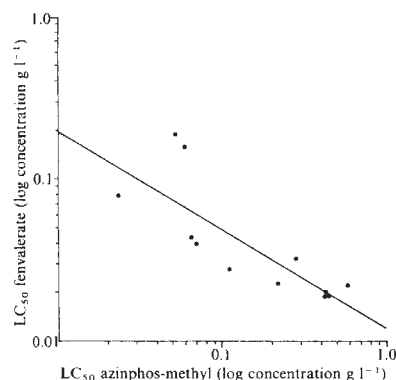


Fig. 1 Relationship between LC₅₀ values of azinphos-methyl and fenvalerate for strains of two-spotted spider mite ($y = 0.011x^{-0.623}$; $r = 0.804$; $P > 0.01$).

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Micromonas pusilla is an extremely small (1.5 to 3.0 μm) photosynthetic flagellate which occurs widely in both coastal and oceanic waters and is occasionally present in large numbers

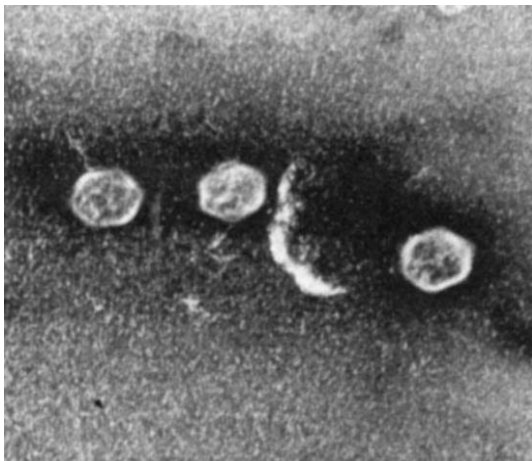


Fig. 1 Transmission electron micrograph of negatively stained MPV particles in clarified lysate from a culture of *M. pusilla*. $\times 76,500$.

(10^3 – 10^5 cells l^{-1})⁴. It is therefore a frequently overlooked but important member of the marine nanoplankton. *Micromonas pusilla* is usually considered to be an anomalous member of the Prasinophyceae^{5,6}. Although plant-like in that it is photosynthetic, it lacks a cell wall.

During a transmission electron microscope (TEM) study of natural communities of nanoplankton in the Strait of Georgia, British Columbia, in April 1975, polyhedral VLPs were observed within *Micromonas* cells from an enrichment culture of wild, mixed British Columbia nanoplankton. It was noted that *M. pusilla* disappeared rapidly from the enrichment culture and that medium from such a contaminated culture, after filtration through a 0.22- μm Millipore membrane filter, could cause lysis of a previously healthy unialgal culture. This infective filtrate caused the destruction of cultures of four unialgal strains of *M. pusilla* originating from both Atlantic and Pacific Oceans and maintained in the Northeast Pacific Culture Collection in the Department of Oceanography at the University of British Columbia.

Medium from lysed *M. pusilla* cultures was spun at 10,000g for 30 min to remove cell debris and negatively stained with 2% uranyl acetate. TEM examination of the clarified lysate revealed particles with hexagonal or pentagonal outlines measuring 130–135 nm in diameter (Fig. 1). No tail-like structures or other external appendages were observed.

The clarified lysate was inoculated into actively growing cultures of *M. pusilla* (whose cells, when examined with TEM,

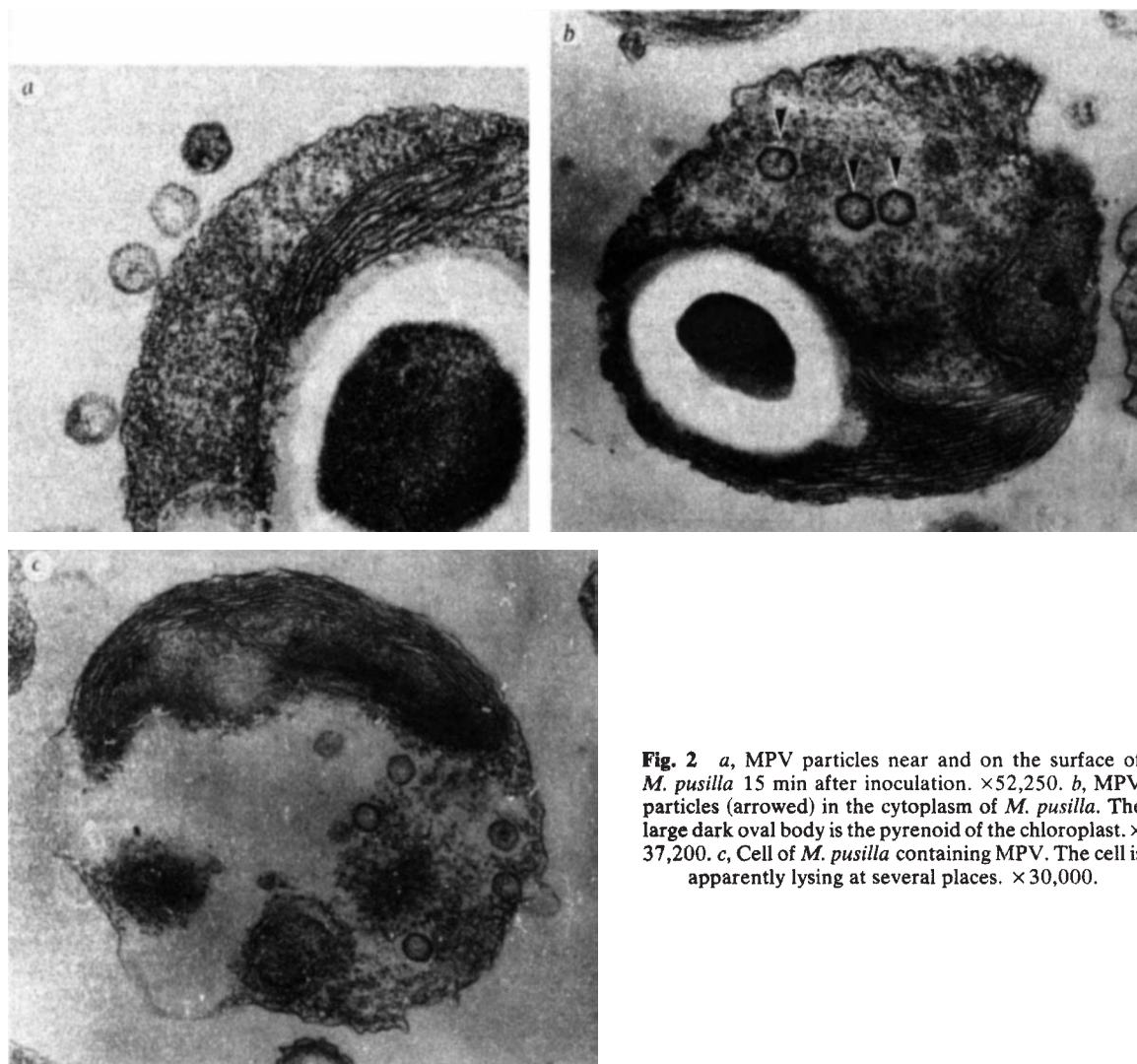


Fig. 2 *a*, MPV particles near and on the surface of *M. pusilla* 15 min after inoculation. $\times 52,250$. *b*, MPV particles (arrowed) in the cytoplasm of *M. pusilla*. The large dark oval body is the pyrenoid of the chloroplast. $\times 37,200$. *c*, Cell of *M. pusilla* containing MPV. The cell is apparently lysing at several places. $\times 30,000$.

did not exhibit particles). Ultrastructural examination of these cultures indicated a predictable series of events. (1) Within 15 min polyhedral particles adhere to host cell surfaces (Fig. 2a). This localised attachment is followed by the fusion of adjacent host and particle surfaces, establishing confluence between the particle core and host cytoplasm. Particle entry by host phagocytosis (virophexis) was never observed. (2) Early stages of interaction described above are followed by an 'eclipse period' of approximately 3 h. Cells examined during this interval demonstrate no ultrastructural peculiarities. (3) Progeny particles appear within the cytoplasm (Fig. 2b). Their appearance is associated with the production of large amounts of scattered cytoplasmic fibrils (~5–8 nm in diameter) and clusters of small, membrane-bound vesicles never present in 'healthy' cells. Particles were never seen within the nucleus or associated with specific cell organelles. The *in situ*, sectional views of the particles are similar in shape and size to those observed in negatively stained preparations of the isolated particles. (4) Progeny particles are released by localised rupture of the cell membrane, often seen at several locations in the same cell, the cell contents including the particles being released into the medium (Fig. 2c).

Regularly shaped, polyhedral, intracellular structures, first observed in a naturally occurring population of *M. pusilla*, have been isolated and described. We conclude that the following evidence is sufficient to establish the viral nature of these entities. First, the particles can be introduced into and cause lysis of four geographical isolates of *M. pusilla*. Second, the particles pass through a 0.22- μ m filter. This filtrate causes lysis as described above. Third, the morphology of these particles is similar to that of known viruses, and finally, the location of the particles relative to the time from introduction until cell lysis is consistent with that of a viral infection.

The particles have been tested with 46 planktonic algal isolates representing 35 species from 5 classes. Lysis occurred only in the four isolates of *M. pusilla*. The virus has been designated '*Micromonas pusilla virus*' (MPV).

This is the first case of controlled viral destruction of a marine phytoplankton organism known to us. Freshwater cyanophyte phytoplankters have been reported to be destroyed by 'cyanophages'⁷. The infection of these walled prokaryotic hosts resembles bacteriophage activity and is quite different from that of the small naked eukaryotic cells described here. The mode of entry of MPV seems unlike any virus previously described. MPV also differs from other algal viruses in that penetration through a cell wall is not required. As the virus is known to occur in natural seawater, at least in British Columbia and neighbouring Washington state⁸, the ecological effect of MPV virus on the population dynamics of *M. pusilla* should be investigated.

As characterisation of MPV has not been completed, the group of viruses to which it belongs is unknown. On the basis of its large size, general morphology and apparent cytoplasmic site of assembly, MPV resembles a large number of viruses which have been referred to collectively as icosahedral cytoplasmic deoxyriboviruses^{9,10} or the Iridoviridae¹¹. This group of viruses is characterised in part by the presence of a DNA genome and by the iridescent appearance of crystals formed within their multicellular hosts or pellets of centrifuged virus. The definitive relationship of this newly identified viral pathogen with known virus groups can be determined only after information on its biochemical and biophysical properties becomes available.

Rapid deposition of subcutaneous areolar tissue in response to sexual stimulation in mice

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Many of the reproductive processes of house mice are regulated exteroceptively by social cues¹. Puberty in a female mouse, for example, is a labile event that can be accelerated markedly if the young female is housed with an adult male². The relevant male stimuli in this case are a combination of pheromonal and tactile cues^{3–5}. The female's responses to these cues include an immediate, sequential release of gonadotropic and ovarian hormones⁶, which in turn evoke a series of predictable changes in most of the female's reproductive tissues and culminate in the pubertal ovulation. I report here an unexpected consequence of exposing a young female mouse to a male, namely an intense response of a non-reproductive tissue. Specifically, young female mice experience a rapid thickening of their subcutaneous areolar tissue when they are exposed to males. The proximal cause of this response seems to be largely, but not exclusively, an enhanced secretion of ovarian oestrogen.

The phenomenon is illustrated in Fig. 1. In these preliminary experiments, CF1 female mice were weaned at 21–24 d and immediately housed in a room free of males. When they weighed 16–17 g, the females were either regrouped in the same room or paired individually with adult males in another room, a standard procedure for studying the socially induced acceleration of puberty in mice^{2,6}. All females were killed after 3 d of such housing. Samples of skin (1 cm²) were removed from the back of each female, fixed in formalin and stained with haematoxylin and eosin. The examples presented in Fig. 1 are typical of the results. The subcutaneous tissue in all 10 females exposed to males was 5–20 times thicker than that of any of the 10 control females. This thickening seemed to occur under the skin on all parts of the body but was studied in detail only on the back.

Many types of connective tissue are known to be sensitive to steroids of ovarian and/or adrenal origin^{7,8}, although the areolar tissue under the skin seems to have been routinely ignored in such studies⁹. A second experiment therefore examined the possible role of the ovary as a mediator of the socially enhanced deposition of areolar tissue. A group of 16 females (15–16 g) was ovariectomised or subjected to sham operations. Following 3 d of recovery, these females were either paired with males or housed in groups in a room free of males. Dorsal skin samples

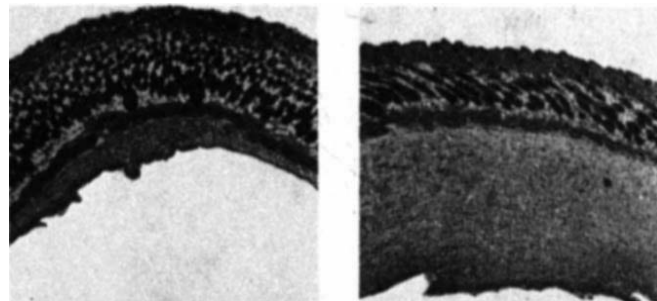


Fig. 1 Sections of skin taken from the backs of young female mice after the animals had been kept in groups in a male-free room (left) or individually paired with a male for 3 d (right). The thicker layer of subcutaneous connective tissue underlying the skin in the section on the right is obvious. The difference in thickness of the cutaneous layers themselves is not related to any experimental variable; such variation between individuals is normal for this stock of mice.

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Table 1 Changes in thickness of subcutaneous areolar tissue and mean uterine weight in young female mice following ovariectomy

	Areolar tissue (μm)		Uterine weight (mg)	
	Ovx	Sham	Ovx	Sham
Male-exposed	142 $\pm$ 7	1,602 $\pm$ 111	10 $\pm$ 1	115 $\pm$ 8
Grouped	99 $\pm$ 2	109 $\pm$ 8	11 $\pm$ 1	23 $\pm$ 3

The female mice were ovariectomised (Ovx) or sham operated and then either exposed to males or held in groups for 4 d ($n = 4$ in each case). The results are the mean $\pm$ s.e.

were collected 4 d later and sectioned on a cryostat; the thickness of the areolar tissue was estimated in repeated samples with a micrometer eyepiece. The results in Table 1 show an average 15-fold increase in the thickness of this tissue in those sham-operated females exposed to males. This response to a male was largely but not entirely eliminated by ovariectomy. Table 1 also records some basic differences between subcutaneous connective tissue and the uterus with regard to their relative responsiveness to ovariectomy and male stimulation. Exposing a young female mouse to a male results in a sequential release of luteinising hormone from the pituitary and oestradiol from the ovary⁶. The uterus gains weight rapidly in response to the enhanced secretion of oestradiol and, as shown in Table 1, ovariectomy completely abolishes such male-induced growth of the uterus but is not completely effective in blocking the deposition of subcutaneous areolar tissue in response to male stimulation. Furthermore, the uterus is markedly and significantly ($P < 0.01$) smaller 4 d after ovariectomy whereas the increase in the thickness of subcutaneous areolar tissue at this time is not significantly depressed by ovariectomy.

The results of a series of pilot studies, each of which involved the injection of steroid or pituitary hormones, suggested that the female mouse's subcutaneous connective tissue is strongly responsive to oestradiol but not to several other hormones, including progesterone and ACTH. Figure 2 shows a dose-response curve of areolar tissue thickness in young females implanted with various doses of oestradiol. These females were ovariectomised at 15–16 g and allowed 3 d to recover, after which they were implanted with hormone. Oestradiol implants were made by mixing 17 β -oestradiol with Silastic adhesive (Type A, No. 891, Dow Corning). The resulting suspension was packed in a 10-mm Silastic capsule (i.d. 0.04 mm; o.d. 0.085 mm, Dow Corning). A suspension of 0.1 mg oestradiol

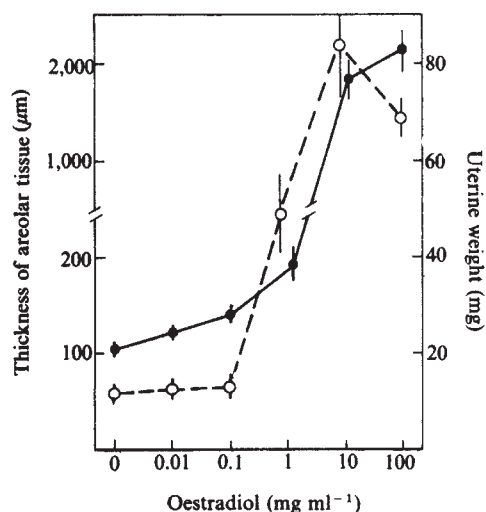


Fig. 2 Mean thickness of subcutaneous areolar tissue ($\mu\text{m} \pm \text{s.e.}$) (●) and mean uterine weight ($\text{mg} \pm \text{s.e.}$) (○) in young female mice that were ovariectomised and implanted with Silastic capsules containing oestradiol.

per ml resulted in a significant thickening of the areolar tissue ($P < 0.01$) although the uterus was unresponsive to this dose of oestradiol. Both the uterus and areolar tissue responded strongly to doses above 0.1 mg ml⁻¹. With 10 mg ml⁻¹ there was slightly more deposition of areolar tissue than occurred after 4 d of male exposure in an intact female (see Fig. 2 and Table 1).

What might be the adaptive function of the deposition of this layer of subcutaneous tissue in response to a male? This layer of connective tissue does not anchor the skin to the underlying musculature in the mouse, as it does in some other mammals¹⁰. Despite the total absence of fat cells in this tissue when under the skin of the back (Fig. 1), it is impregnated with fat cells in the area of the mammary glands, but the preponderance of such cells does not seem to change at puberty. Thus, one would not expect the pubertal increase in this layer of connective tissue to be important for post-pubertal steroid metabolism or thermal insulation. One possibility is that, as this layer of connective tissue envelops the mammary glands ventrally, its thickening in response to oestrogen could be a provision for the support of these glands when they enlarge during late pregnancy, and could subsequently serve to anchor them firmly to the skin during lactation. Whatever the function for this tissue response, however, the present results do show that in the mouse this tissue is an important target of oestrogen action, and is perhaps even more sensitive to oestrogen than the organ best recognised as a target for this steroid, the uterus.

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Resistance of mice suppressed for IgM production to *Babesia microti* infection

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The immunological mechanisms responsible for overcoming infections with *Babesia*, an intra-erythrocytic protozoan, are not fully understood. Although high titres of specific anti-babesial antibodies have been observed in several species of animals¹, and protection has been obtained by transfer of large volumes of recovery serum², the role of antibody in the immune response to an infection is uncertain. The present study investigates the nature of B-cell participation during *Babesia microti* infections by observing the course of the disease in mice in which IgM production has been suppressed from birth and which contain no B cells. The results show that, in contrast to control mice, which develop and subsequently clear circulating parasitaemias, suppressed mice show an unexpected resistance to infection as reflected by a virtual absence of parasites in the peripheral circulation.

Table 1 Comparison of parasitaemias in IgM-suppressed and control mice following infection and challenge with *B. microti* (expt 1)

Mice	Days after initial infection										
	5	8	11	13	15	17	19	22	24	60*	67
IgM Suppressed	0	0	0	0		0	<0.2		9.6	D†	D
	0	0	0	0		0	<0.2		<0.2	0	0
	0	0	0	0		0	0		0	0	17.6
	0	0	0	0		0	0		0	0	<0.2
	0	0	0	0		0	0		0	0	0
	0	0	0	0		D	D		D	D	D
	0	0	0	0		0	0		<0.2	0	0
	0	0	0	0		0	0		D	D	D
Anti-ferritin antibody treated	0	<0.2	5.8	5.4	0.2	3.4	1.0	<0.2	0	<0.2	0
	0	<0.2	2.8	4.6	1.2	<0.2	0.6	<0.2	0	0	0
	<0.2	<0.2	22.4	24.4	8.4	3.4	4.8	0.8	<0.2	<0.2	0
	0	<0.2	5.6	0	ND	ND	<0.2	<0.2	0	0	0
	0	<0.2	9.2	11.6	8.0	3.6	0.8	<0.2	<0.2	0	0
Untreated normal controls	0	3.6	26.0	9.6	4.6	<0.2	1.2	<0.2	0	0	0
	0	4.0	11.6	12.0	10.0	9.0	4.0	0	<0.2	0	0
	0	8.6	41.6	14.2	9.0	4.2	4.8	<0.2	<0.2	0	0
	0	3.8	18.0	20.8	10.2	6.8	5.4	<0.2	<0.2	0	0
	0	10.2	17.0	6.2	4.4	0.8	1.8	<0.2	0	0	0
	<0.2	4.6	11.2	12.4	6.8	3.2	5.4	<0.2	<0.2	<0.2	0
	0	0.4	12.0	6.2	6.0	1.8	3.2	<0.2	0	0	<0.2

Three-month-old female B6CF₁ mice injected from birth with either G α MIgM (group 1) or goat anti-ferritin antibody (group 2), or untreated (group 3), were infected i.p. with 10⁶ *B. microti* and per cent parasitaemias for each mouse determined by Giemsa stain. Suppressed mice were still negative for circulating IgM when tested by Ouchterlony analysis 29 days after infection. Removal of suppression at day 30 did not result in the appearance of parasites in peripheral smears 7 or 14 days later. On day 37, blood samples from the five remaining suppressed mice were subinoculated into B6CF₁ normal mice. Three of the recipients had low parasitaemias at day 12 after sub-inoculation.

*Homologous immunity was tested on day 53 by infection of all groups with 10⁶ *B. microti* and smeared on day 7 (D60) and day 14 (D67) thereafter. Six months after challenge, groups 1 and 2 received 10⁶ *P. yoelii* i.p. 90% Of mice died within 10 days. ND, not done; D, died.

†Died day 29.

The following three age-matched groups of (C57BL/6 $\times$ BALB/c)F₁ (B6CF₁) and BALB/c $\times$ C57BL/6)F₁ (CB6F₁) mice, each containing 4–10 animals, were intraperitoneally (i.p.) infected with 10⁶ *B. microti* (Kings 67 strain): (1) mice suppressed from birth with a globulin fraction of goat anti-mouse IgM (G α MIgM)³, and which showed no circulating IgM at the time of infection (suppressed group); (2) mice which received a globulin fraction of goat anti-ferritin antibody in a similar injection regime, to control for any effects of repeated antibody injections (antibody controls), and (3) normal, untreated mice (normal controls). Parasitaemias, determined by Giemsa stain of thin smears from tail blood, were first examined at various times after primary infection with *Babesia*, again in the succeeding interval during which the IgM suppression was discontinued, and finally, following challenge with the same parasite. Thereafter, immunity to a heterologous parasite was tested by infection with 10⁶ lethal malaria (17 $\times$ *Plasmodium yoelii*). The data from two such experiments, which compare the per cent parasitised erythrocytes observed in the periphery of suppressed versus both control groups, are presented in Tables 1 and 2.

Results from the first experiment, which used 3-month-old B6CF₁ female mice throughout, are shown in Table 1. As can be seen, in contrast to the rise and fall in parasitaemias observed in either control group, the suppressed group showed a virtual absence of parasitised red blood cells (RBC) in the circulation (except for one mouse which had 9.6% of cells infected). Although this apparent lack of parasites continued when mice were released from suppression, low numbers must have been present, as three out of five of the remaining suppressed mice were positive on sub-inoculation 37 days after infection. Despite the fact that parasites were rarely seen in the suppressed mice following primary infection, these animals nevertheless seemed to be as well protected as control mice against homologous challenge 7 weeks later. In addition, both the suppressed and control groups exhibited a lack of heterologous immunity after infection with lethal *P. yoelii* several months after primary infection with *B. microti*. These findings are similar to those of Cox using non-lethal *P. yoelii*⁴.

It is possible that the coating of parasitised cells in recipient mice by G α MIgM either directly, or indirectly through passively transferred IgM on infected cells, could have resulted in blocking of infection by parasites or rapid clearance of infected cells by macrophages in the suppressed mice. To test for such effects, the parasitised cells used in the second experiment were washed three times in saline before infection and, in addition, a portion of these washed cells was incubated in G α MIgM serum for 30 min at 4°C before injection into normal mice, to examine whether cells deliberately coated with Ig were rendered non-infectious. In this experiment, 2-month-old B6CF₁ mice (suppressed and antibody control groups) and CB6F₁ mice (normal group) of both sexes were used, and four normal 4-month-old female B6CF₁ mice also served as controls.

The results, presented in Table 2, are similar to those obtained in the first experiment in that (1) suppressed mice showed almost no sign of parasites compared with the controls, (2) removal of suppression had no effect on the levels of parasitaemia, and (3) the degree of protection on challenge was similar in each group. The differences in parasitaemia between the antibody and normal control groups which occurred in the first experiment were not seen in experiment 2. The lower peak of parasitaemias seen during the second experiment, however, probably reflects the fact that these mice were 4 weeks younger than those used in experiment 1, as the 4-month-old normal control mice developed much higher parasitaemias with the same inoculating dose. These results also suggested that at least in the younger mice, a sex-related susceptibility to infection with *B. microti* exists, as male mice always had higher parasitaemias than females. Furthermore, the preincubation of the parasitised cells in G α MIgM did not affect the ability of the parasite to infect and multiply, but rather resulted in consistently higher parasitaemias (Table 2). As in experiment 1, all mice were equally protected following reinfection with *B. microti*, although low parasitaemias in recipients of sub-inoculated blood from all groups indicated that low levels of infection persisted after homologous challenge. As an additional control for the effects of G α MIgM injections, an experiment was done in which normal adult

B6CF₁ mice were given the antisera for 8 days before and 22 days after infection with 10⁶ *B. microti*. These mice developed parasitaemias similar to those seen in normal control mice.

Previous studies have suggested that an intact complement system is essential for the development of Babesial parasitaemias^{5,6}, and more recently, Chapman and Ward⁷ demonstrated that factors of the alternate pathway as well as the third (C3) and fifth (C5) components from human or rat serum are required for *Babesia rhodani* penetration of human red cells in

explained by increases in the activity of natural killer (NK) cells^{11,12} or macrophages in the suppressed mice. A protective role for such cells, however, is not compatible with the studies of Weinbaum *et al.*¹³ which demonstrate the ability of another non-lethal parasite, 17X *P. yoelii*, to infect and eventually kill similarly suppressed mice. It is not known whether the mechanisms preventing a primary parasitaemia are the same as those which result in protection on challenge. Thus, although IgM suppression could explain the lack of parasitaemias follow-

Table 2 Comparison of parasitaemias in IgM-suppressed and control mice following infection and challenge with *B. microti* (expt 2)

Mice	Sex	No. per group	7	9	12	13	14	19	35	111*	121
IgM-suppressed B6CF ₁	M	6	0	0	0	0	0	0.007 (0-0.02)	0.003 (0-0.02)	0	0
	F	5	0	0	0	0	0	0.08 (0-0.4)	0	0	0
Antibody control B6CF ₁	M	4	0.015 (0-0.02)	1.9 (0-3.6)	9.2 (0.02-15.4)	11.1 (0-22.6)	5.4 (0-14.8)	0.17 (0.02-0.6)	0	0	0
	F	9	1.2 (0-5.4)	6.6 (0-17.4)	4.7 (0-11.6)	2.8 (0-12.2)	1.1 (0.2-5.6)	3.2 (0.2-2.04)	0	0	0
Normal control CB6F ₁	M	4	0.3 (0-0.6)	4.1 (0-15.2)	9.2 (0.2-31)	4.3 (0.2-14.8)	2.7 (0.4-4.6)	7.3 (0.02-26.8)	0	0	0
	F	5	0.13 (0.02-0.6)	0.32 (0.02-0.8)	3.5 (0.02-11.4)	0.93 (0-2.4)	0.28 (0-0.8)	0.008 (0-0.02)	0.005 (0-0.02)	0	0
†Normal control CB6F ₁	M	3	0.27 (0.02-0.6)	7.4 (4.8-10.4)	16.5 (9.6-23)	12.3 (20-27.6)	7.2 (2.6-14.4)	0.4 (0-0.6)	0	0	0
	F	3	0.02 (0.02)	2.7 (0.2-5.4)	4.1 (2.6-5.4)	0.28 (0.02-0.6)	0.74 (0.02-2)	0.07 (0.02-0.2)	0	0	0
‡Normal control B6CF ₁	F	4	0.26 (0.02-0.4)	6.9 (3.8-10)	25.3 (14-46.8)	22.8 (17.2-30.8)	18.5 (11-21.8)	3.5 (0.6-7)	0	0.005 (0-0.2)	0.01 (0-0.4)

Male and female 2-month-old B6CF₁ mice (IgM-suppressed and antibody control groups) and CB6F₁ mice (normal controls) were infected with 10⁶ *B. microti* i.p. and parasitaemias determined by Giemsa stain. Data are expressed as mean per cent parasitaemia in each group. The range for each group is given in parentheses. All parasitised RBC were washed three times in saline before injection.

* All groups were challenged on day 103 with 10⁶ *B. microti*.

† Washed parasitised RBC were preincubated in GαMIgM for 30 min at 4 °C before injection.

‡ Four-month-old normal B6CF₁ controls.

vitro. As it was possible that the absence of parasitaemia in IgM-suppressed mice reflected a deficiency in the required complement components, serum samples from these animals were tested for C3 activity by immune adherence⁸. This assay uses the fact that human erythrocytes which have a receptor for the third component of complement (C3b) will adhere to sensitised sheep RBC preincubated in serum containing C3 components (as well as C1, C4 and C2). The results (not shown) indicated that the C3 titres in three suppressed mice were in the same range as those in the three control mice and makes it unlikely that a C3 deficiency could explain the above results.

Based on the present data, the simplest explanation for the apparent lack of susceptibility to infection of suppressed mice is that, to invade mature RBC, *Babesia* parasites require IgM antibody; the absence of the capacity to make IgM antibody thus confers resistance. Although the specificity of such an antibody is not known, an interesting possibility is that an autoantibody with specificity for membrane determinants on parasitised or normal erythrocytes is required for invasion. Antibodies reactive with antigens on enzyme-treated isologous RBC have been demonstrated during protozoal infections^{9,10} and are also elicited following infection of normal mice with *B. microti* (not shown). Perhaps, therefore, red cell antigen-antibody complexes in association with complement facilitate penetration of the parasite into erythrocytes by alteration of the red cell membrane.

However, several other interpretations of the data are possible. For example, because only peripheral smears were examined, parasites sequestered in specific organs would not have been scored. In addition, the observed resistance might be

ing the initial infection, it is possible that T cells primed by the few parasites present during that time are effective in protecting mice after suppression is discontinued¹⁴. For this reason, the present observations probably reflect the existence of several different mechanisms responsible for the protection observed in the IgM-suppressed mice, some of which may be similar to those resulting in the phenomenon of age resistance in young cattle¹⁵.

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Cross-linking of lymphocytic surface immunoglobulin inhibits its production via a cyclic nucleotide mechanism

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When antibody to immunoglobulin (anti-Ig) reacts with Ig molecules on B-lymphocyte surfaces the Ig, tethered by the antibody, moves over the surface to form patches and finally a cap at one pole of the cell. Simultaneously, the Ig-antibody complexes are removed by pinocytosis at the patches and cap, until the surface is cleared of the target molecules^{1,2}. In immunofluorescent studies, Sidman and Unanue³ followed the reappearance of the surface Ig during subsequent culture of the cells. They observed that splenic lymphocytes from adult mice re-express surface Ig promptly, whereas those from neonatal animals re-express poorly or not at all. They suggested that this phenomenon might be related to the greater susceptibility of lymphocytes from immature animals to tolerisation by antigen. Such an interpretation was supported by a simultaneous study⁴ showing that explanted mouse fetal lymphocytes were less able to re-express surface Ig after treatment by anti-Ig than were adult lymphocytes. However, Ault and Unanue⁵ later reported in man a similar dichotomy between blood and nodal lymphocytes, the former re-expressing surface Ig more poorly. We report here studies on guinea pig leukaemic lymphocytes, which have also shown delayed re-expression of surface Ig after clearing by antibody. We present evidence that the delayed re-expression reflects a reduction in the normal delivery rate associated with turnover at the surface. The reduction follows a train of events initiated by cross-linking of the surface Ig and including an increase in intracellular cyclic AMP. Furthermore,

the shutdown in delivery is apparently specific, inasmuch as delivery of two other surface molecules we have been able to quantify proceeds unimpeded. These observations suggest a basic mechanism expressed to varying extents by normal lymphoid populations to account for their differing rates of surface Ig regeneration³⁻⁵.

The L₂C leukaemia arose spontaneously in a female strain 2 guinea pig and has since been maintained by passaging *in vivo* through syngeneic or near-syngeneic animals⁶. Cells for the present work were collected from the blood of animals in the terminal stages of the disease⁷. The cells show a surface chemistry typical of B lymphocytes⁸. The surface Ig is of class IgM λ with a density of approximately 50,000 molecules per cell and a half life on the surface of about 5 h (ref. 9).

The general experimental plan was to perturb the surface Ig of the L₂C cell in culture at 37 °C, by antibody or other means, to chill the cells, remove the perturbing agent and wash, and then to culture the cells again, sampling at intervals to determine the density of the Ig or other surface molecules. The anti-Ig used was raised in sheep against the C₁ determinants of guinea pig Ig, and was purified by affinity chromatography¹⁰. Antibodies to the histocompatibility antigens Ia(2,4) and GPLA(B1) were raised in guinea pigs: anti-Ia by injecting strain 13 animals with normal strain 2 splenic and nodal lymphocytes¹¹, and anti-GPLA by injecting Dunkin-Hartley animals (lacking GPLA(B1) on testing with an antiserum and matching strain 13 in Ia to the extent that testing was possible) with normal strain 13 lymphocytes. Estimations of the densities of L₂C surface molecules were carried out by allowing the cells to react at 4 °C with isotopically labelled antibody. The surface Ig was labelled directly by ¹²⁵I-Fab γ prepared from purified anti-C₁, the Ia and GPLA antigens indirectly by incubating first with IgG from the corresponding guinea pig antiserum and then, after an intermediate washing, with ¹²⁵I-labelled purified sheep anti-(guinea pig Fc γ). Antibody concentrations were on the plateaus of previously defined labelling curves. Labelling of controls by non-antibody Fab γ or IgG was always negligible.

Figure 1a shows that the L₂C surface Ig is turning over with a half life of 5 h: this is demonstrated both by the rate of re-appearance of its Fab regions after their removal by papain (leaving the Fc regions attached to the membrane¹²), and by the

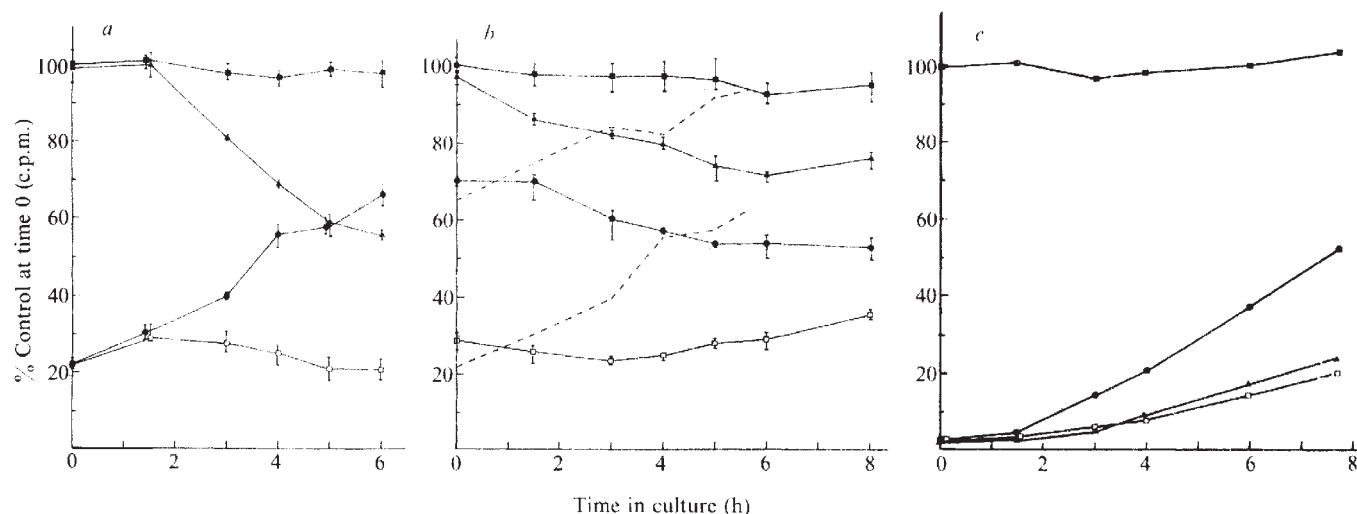


Fig. 1 Densities of surface Ig. After the treatments described below the cells were cultured with swirling at 37 °C in supplemented Eagle's medium (5×10^6 cells ml^{-1}), and were sampled at intervals to quantify the surface Ig by labelling with radioactive anti-Ig. The ranges shown are of triplicates. **a**, Normal turnover. $\blacksquare$, Control cells, treated as for the papain digestion but with the enzyme omitted; $\bullet$, treated with papain 0.1 mg ml^{-1} and dithiothreitol 0.1 mM for 30 min at 37 °C; $\blacktriangle$, treated as the controls, then cultured in the presence of 0.1 mM cycloheximide; $\square$, treated with papain and dithiothreitol, then cultured in the presence of 0.1 mM cycloheximide. **b**, Reduced delivery following treatment with anti-Ig. Cells were exposed to sheep IgG at $50 \mu\text{g ml}^{-1}$ for 30 min at 37 °C before washing and culturing. The sheep IgG contained anti-Ig antibody at the following concentrations: $\blacksquare$, zero; $\blacktriangle$, $1 \mu\text{g ml}^{-1}$; $\bullet$, $8 \mu\text{g ml}^{-1}$; $\square$, $50 \mu\text{g ml}^{-1}$. - - -, Parallel batches of cells treated with papain at 0.01 mg ml^{-1} (upper line) or 0.1 mg ml^{-1} (lower line), without exposure to antibody. **c**, Confirming reduced delivery due to anti-Ig. The sequential treatments were each of the same duration as in **a** and **b**, and were carried out with chilling and washing after each step. $\blacksquare$, Treated with normal IgG $200 \mu\text{g ml}^{-1}$, followed by papain-free buffer; $\bullet$, treated with normal IgG $200 \mu\text{g ml}^{-1}$, followed by papain 0.1 mg ml^{-1} ; $\square$, treated with anti-Ig $200 \mu\text{g ml}^{-1}$, followed by papain-free buffer; $\blacktriangle$, treated with antibody $200 \mu\text{g ml}^{-1}$, followed by papain 0.1 mg ml^{-1} .

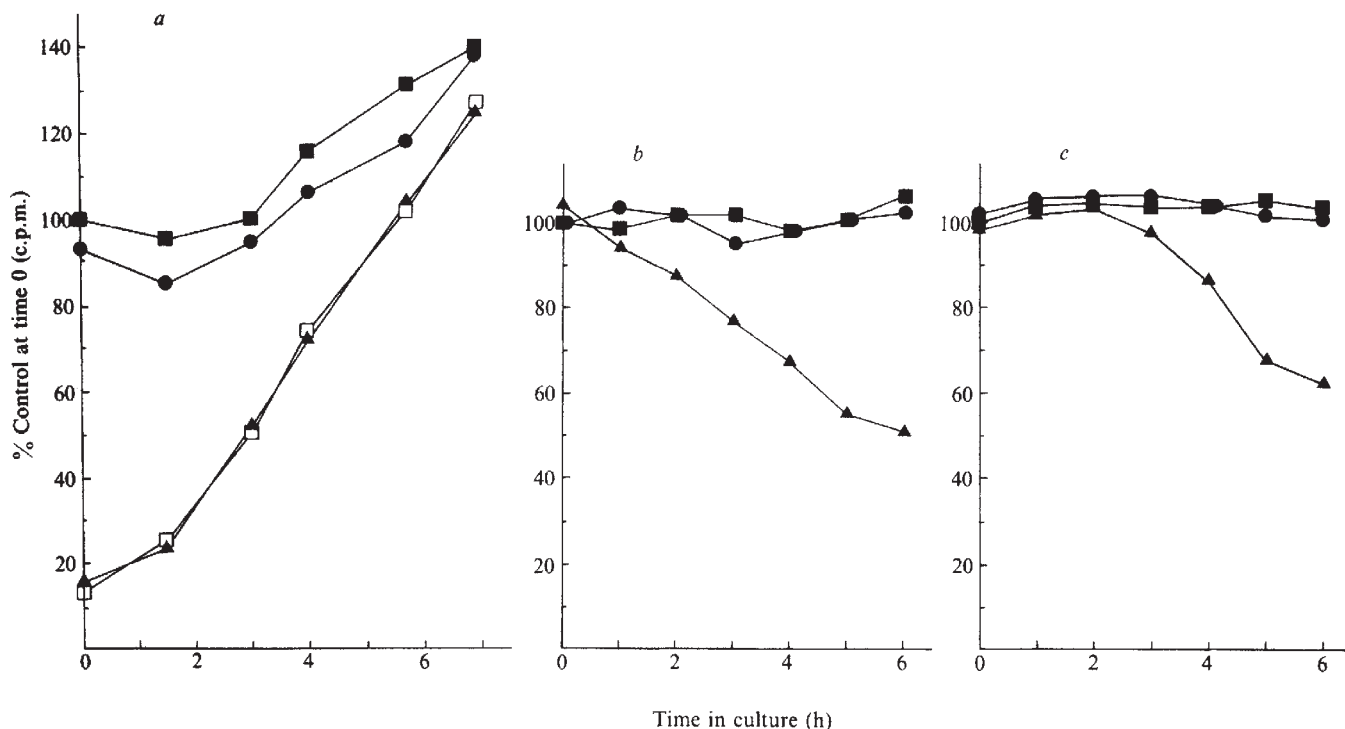


Fig. 2 Densities of surface GPLA and Ia. Ranges are omitted for clarity, but were similar to those shown in Fig. 1. *a*, Delivery of GPLA to the surface following treatments with anti-Ig and/or papain. ■, Treated with sheep normal IgG $200 \mu\text{g ml}^{-1}$, then with papain-free buffer; ▲, treated with sheep normal IgG $200 \mu\text{g ml}^{-1}$, then with papain 1.9 mg ml^{-1} for 30 min at 37°C ; ●, treated with anti-Ig $200 \mu\text{g ml}^{-1}$, then with papain-free buffer; □, treated with anti-Ig, then with papain. *b*, *c*, Densities of surface GPLA and Ia following treatment with anti-Ig or cycloheximide. ■, Treated with sheep normal IgG $200 \mu\text{g ml}^{-1}$; ●, treated with anti-Ig at $200 \mu\text{g ml}^{-1}$; ▲, treated with sheep normal IgG, then cultured in the presence of 0.1 mM cycloheximide.

rate of disappearance of surface Ig after halting protein synthesis with cycloheximide. However, Fig. 1*b* indicates that after extensive clearing of the surface Ig by antibody at 37°C there is no significant regeneration in subsequent culture until after about 4 h (lowermost solid line). Figure 1*b* demonstrates, moreover, that amounts of antibody insufficient to clear the surface completely by capping and pinocytosis (intermediate two solid lines) nonetheless induce a delay in delivery of new surface Ig. For about 6 h after the reaction with antibody the

surface Ig continues to fall, implying that reduced delivery is not keeping pace with shedding. These rates of regeneration are in striking contrast to those shown in the same figure to occur after partial or complete clearing of the surface by papain.

Figure 1*c* shows that the action of antibody and papain sequentially yields the same slow regeneration as occurs after antibody alone. This rules out the possibility that the regeneration rate after antibody is normal but after papain spuriously high due to some stimulatory action of the enzyme. It also

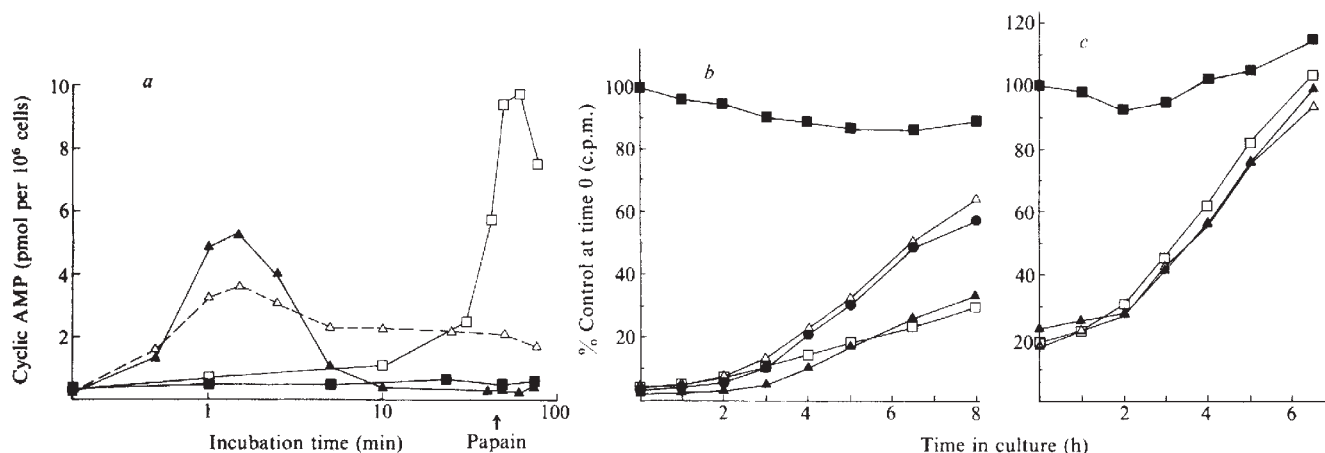


Fig. 3 Delivery of surface molecules correlated with cyclic AMP levels¹⁴. In *b* and *c* the ranges of triplicates were similar to those shown in Fig. 1. *a*, Levels of intracellular cyclic AMP following exposure for 45 min at 37°C to: ■, sheep normal IgG, $200 \mu\text{g ml}^{-1}$; ▲, anti-Ig, $200 \mu\text{g ml}^{-1}$; △, isobutylmethylxanthine (Aldrich), 2 mM; □, cholera toxin (Sigma), 100 ng ml^{-1} . In the last three cases the cells were exposed to papain 0.1 mg ml^{-1} for 30 min at 37°C immediately after exposure to the primary agent, so as to mimic conditions for the experiment depicted in *b*. The treatment with papain in fact made no difference to the levels of cyclic AMP attained, and viability (exclusion of Trypan blue) remained at $>95\%$ in all groups. *b*, Delivery of surface Ig after treatment for 45 min at 37°C with: ▲, anti-Ig, $200 \mu\text{g ml}^{-1}$; △, isobutylmethylxanthine, 2 mM; □, cholera toxin, 100 ng ml^{-1} . After these primary treatments the cells were treated with papain 0.1 mg ml^{-1} for 30 min at 37°C to remove residual surface Ig, and finally washed and cultured to observe regeneration of the surface Ig. Two control groups were exposed to sheep normal IgG, $200 \mu\text{g ml}^{-1}$, as the primary treatment: ●, followed by treatment with papain; ■, not followed by papain. *c*, Delivery of surface GPLA following treatment as in *b*, but with papain at 1.9 mg ml^{-1} to remove residual GPLA.

provides evidence against the possibility that delayed regeneration following antibody treatment is due to chronic clearance by persisting sheep antibody on the surface, because this antibody would itself have been susceptible to proteolysis by papain.

In experiments in which the cells were allowed to react with monovalent Fab γ fragments of antibody, no effects were observed on the density of the surface Ig, whereas F(ab γ)₂ fragments had the same effect as whole antibody. Thus, it seems that a necessary signal for delayed delivery is cross-linking of the surface Ig, with no apparent role for the antibody Fc region.

The experiments shown in Fig. 2 indicate that anti-Ig reacting with L₂C cells had no effect on the delivery of two non-Ig molecules, GPLA and Ia, to the cell surface. Figure 2a shows that the rate of regeneration of GPLA following its removal by papain is independent of the cell being exposed to anti-Ig. (The rising control values were often seen with this antigen, and might be related to the cell cycle¹³.) A similar experiment could not be carried out for Ia, which could not be removed by any of a series of proteolytic enzymes tested. However, Fig. 2b and c show, respectively, that neither GPLA nor Ia underwent any diminution in density after the cells had been treated with anti-Ig, contrary to the result expected if delivery of these molecules were impeded. To confirm that both molecules are, in fact, turning over at the cell surface, the same graphs show their rates of fall-off when protein synthesis is halted by cycloheximide: the rates are apparently similar, but with a delay of about 2 h before the Ia starts to fall off, presumably reflecting the size of an intracellular pool.

There is some evidence of the pathway leading to suppression of Ig delivery. Previous work has shown that anti-Ig acting on the L₂C surface triggers a surge in cyclic AMP within the cell¹⁴. Protein kinases will thereby be activated, to phosphorylate other enzymes and possibly finally to yield reduced Ig delivery, among other effects. Such an interpretation is supported by the experiment shown in Fig. 3. Cholera toxin, binding to different receptors (ganglioside *g_{M1}*) on the surface¹⁵, caused after a lag period a marked increase in cyclic AMP and then a reduced delivery of Ig but not of GPLA. The effect was strikingly similar to that given by anti-Ig. Another means of raising the cyclic AMP level, slowing its breakdown by the phosphodiesterase inhibitor isobutylmethylxanthine, had no detectable effect on delivery to the surface. Note, however, that the rise in cyclic AMP caused by this agent can be expected to occur throughout the cell rather than to be concentrated under the plasma membrane, a difference likely to give different sequelae¹⁶.

Two lines of evidence suggest that antibody need only cross-link surface Ig to generate the signal which culminates in reduced Ig delivery. First, clearing by capping and pinocytosis is not necessary (Fig. 1b). Second, the surge in cyclic AMP is evident within 30 s (ref. 14), too short a time for any obvious interiorisation of antibody-Ig complexes². Nevertheless, we cannot entirely rule out a need for at least minimal pinocytosis of the complexes.

We intend to apply the techniques used in this work to investigate other neoplastic lymphocytes, in particular, those of human chronic lymphocytic leukaemia. Clearly, caution must be exercised in extrapolating the behaviour of neoplastic cells to that of their normal counterparts. However, in the present case there is already evidence that the basic phenomenon is expressed to different degrees in normal cells^{3-5,17}. Under such circumstances neoplastic cells, by virtue of their availability and homogeneity, offer considerable scope for elucidating mechanisms and perhaps for providing models of the normal stages of differentiation.

The perturbation of surface Ig in leukaemic lymphocytes by anti-Ig also has therapeutic implications. On these surface molecules there are idiotypic determinants, essentially unique to the clone of cells bearing them, and anti-Ig directed against these particular determinants may prove therapeutically useful¹⁸.

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Human T lymphocyte clones reactive in primed lymphocyte typing and cytotoxicity

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The human HLA-D region, the presumed evolutionary homologue of the H-2 I region in mouse and similar genetic regions in other species¹, codes for multiple antigenic determinants, a finding consistent with the existence of multiple loci^{2,3}. One method developed to define D region encoded determinants is primed LD typing (PLT) wherein cells of one individual are primed *in vitro* in a mixed leukocyte culture (MLC) to allogeneic HLA-D encoded determinants associated with one haplotype^{4,5}. The PLT cells give a rapid proliferative response when restimulated in secondary culture with either the priming cells or third party cells that carry the priming antigen(s). However, as in many cases a PLT cell is probably primed to several determinants, different restimulating cells from unrelated individuals will engender a range of responses varying from very high to very low, presumably depending on the number of determinants shared (or cross-reactive) between the priming and restimulating cells. Such results are difficult to interpret in terms of defining antigenic determinants on the cells of various individuals. Fathman and Hengartner⁶ have circumvented this problem in mouse by cloning murine T lymphocytes from multiply restimulated PLT cells; the progeny of any one clone give highly specific proliferative responses. We report here the use of the supernatant of phytohaemagglutinin (PHA)-stimulated mixtures of human allogeneic cells^{7,8} (designated T-cell growth factor or TCGF) to grow 'clones' of human T lymphocytes following alloactivation in MLC. Clones that give PLT-type responses do so in a highly antigen-specific manner. Following cloning, cells in many cases have undergone 25 or more divisions, thus yielding in excess of 1×10^8 progeny.

All cells to be cloned were alloactivated in primary MLC and on day 4 the blast cell fraction was obtained by unit gravity sedimentation. These blast cells were either cloned, as given below, or recultured and restimulated with the sensitising alloantigens one or more times and then used 2 days after

Table 1 Proliferative response of non-cloned, PLT cells against agarose 'cloned' alloactivated cells

Stimulators	Responders			
	AB _x PLT	Clone A31	Clone A17	Clone A11
HTC:				
DW1	475	NT	575	NT
DW2	708	215	711	506
DW3	478	480	696	604
DW4	281	NT	822	NT
DW5	824	NT	576	NT
DW6	1,031	368	3,031	NT
DW7	2,436	15,684	12,949	980
DW10	588	NT	674	NT
A	148	215	491	355
B	1,424	5,794	2,527	458

Ficoll-Hypaque purified peripheral blood lymphocytes from individual A (10^7) were mixed with an equal number of (4,000 rad) irradiated cells of individual B (AB_x) in Falcon 3013 flasks in 15% heat-inactivated human serum in RPMI-1640 with 25 mM HEPES (15% HS-RPMI). Following 1g sedimentation¹² on day 4 the 'blast' cell containing fractions were pooled and $2.5-5 \times 10^5$ cells in 1 ml 15% HS-RPMI mixed with 1 ml of TCGF containing agarose (Sea Plaque Agarose) to yield a predetermined, optimal concentration of TCGF and a 0.36% agarose concentration. This mixture was placed on a pre-gelled (2.5 ml) layer of 0.5% agarose in TCGF containing media in a 60-mm Petri dish, allowed to gel, and incubated in humidified 5% CO₂ in air at 37 °C until colonies of cells containing 20–100 cells appear. TCGF was always used at predetermined, optimal concentrations with 15% HS-RPMI (TCGF media) and was either prepared as described by Morgan *et al.*⁷ or purchased as pooled units (Associated Biomedic Systems). Colonies were transferred after 7–14 days to round-bottom microtitre wells (Linbro 76-011-05) containing 0.15 ml of TCGF media and irradiated (4,000 rad) A or B lymphocytes as feeder cells ($5-10 \times 10^4$ per well). On days 5–6 of culture 50 µl of TCGF media is added to each well and after an additional 2 to 5 days of culture the amount of ³H-thymidine incorporation was determined in 20-µl samples of each well. The contents of wells with dividing cells were transferred to 16 × 100 mm culture tubes or 16 × 17 mm wells (Linbro 76-033-05) containing TCGF media and $5-10 \times 10^5$ irradiated A or B feeder cells. Thereafter the pre-clones ('clones') were maintained at less than 10^6 cells per ml, replenished with fresh TCGF media every 2–4 days, and resupplied with feeder cells approximately every 10 days. Clones to be tested for PLT response (after washing to remove TCGF) as well as 10 day AB_x PLT cells⁴ were placed at a concentration of 2.5×10^4 cells per well in microtitre V-bottom plates (Linbro 76-021-05) containing 5×10^4 irradiated stimulating cells in 15% HS-RPMI and after 40 h the cultures were labelled with ³H-thymidine for 8 h (A response is easily detectable at 24 h and peaks at 48 h). Values given are mean counts per minute of triplicate samples. NT, not tested.

restimulation for cloning. Cloning was attempted by two different methods. First, cells obtained from a primary MLC were placed in the presence of TCGF, in soft agarose and 7 to 14 days later 'colonies' were removed and subsequently grown in microtitre wells in the presence of irradiated (feeder) lymphoid cells. Second, lymphocytes or cells from blast fractions from a primary MLC were placed at limiting dilutions in the wells of Terasaki microtitre plates in TCGF-containing medium also in the presence of feeder cells. One cannot be sure that the progeny obtained by either of these two methods are true 'clones' until recloning is done; although we have not, as yet, attempted re-cloning, for simplicity of expression we shall refer to the populations generated as clones.

Clones, obtained from MLC blast cells plated in agarose, were tested for reactivity to homozygous typing cells for the presently recognised HLA-DW clusters (Table 1). Of the clones that gave a proliferative response, all did so to the DW7 homozygous typing cell, as did the non-cloned AB_x PLT cells which were collected and tested following the normal sensitisation time of 10 days^{4,5}. In addition, some clones showed a weaker, but highly significant, response to DW6. Assuming that weak stimulation by the DW6 cells represents antigen sharing or cross-reactivity between DW7 and DW6, one would expect to find some clones

that detect this 'shared' determinant; other clones should be specific for DW7. The results shown demonstrate that this is the case. In addition, these data provide evidence suggesting that HLA-D region-encoded determinants are important in the restimulation of the cloned PLT cells.

One of the 'clones' in Table 1 did not show any proliferative response to any restimulating cell tested. Cells of this clone were cytotoxically active against the original sensitising cell but not to targets autologous with the responding cells (Table 2). Agarose-cloned cells grown in TCGF are primarily T cells, 92% of which form sheep red blood cell (SRBC) rosettes while 64% of the non-cloned, AB_x PLT cells (Table 1) rosette with SRBC.

Table 3 shows results obtained with cells of a number of different 'clones' obtained from a different primary MLC in terms of their PLT response either to restimulating cells from the sensitising cell donor, B, or to restimulating cells from individuals C and D, unrelated to B and the donor of the responding cells. Results obtained with the original PLT cell,

Table 2 Cytotoxicity of cells from agarose clone A11 derived from alloactivated MLC blast population

E:T	% Cytotoxicity (±s.d.) on target	
	A	B
20:1	−8.4 ± 9.3	43.1 ± 11.0
5:1	NT	6.0 ± 7.4
1.25:1	NT	−3.6 ± 8.3

'Clone' A11 cells (see footnote to Table 1) were mixed at the given ratio with 1×10^4 ⁵¹Cr-labelled target lymphocytes of the original responder (A) or of the original stimulator (B). After 8 h the amount of ⁵¹Cr released was determined and per cent cytotoxicity calculated based on spontaneous and maximum release from the target cells in medium.

AB_x, indicate that cells of C restimulate to a significant degree, although much less than do those of B; cells of D do not restimulate. Once again, assuming antigen sharing or cross-reactivity between B and C, one would expect to find clones, such as those represented by 40-8E and 40-4E, which give a highly significant response to restimulating cells of either B or C with still no response to cells of D. Other 'clones', such as 40-10E and 40-7E, retain specificity only for B.

Response patterns of cells of these clones allow definition of HLA-encoded antigens with a degree of specificity previously unobtainable with the average PLT or homozygous typing cell reagent. Low levels of cross-stimulation by third party test cells of non-cloned, normal PLT reagents, a finding that has been very difficult to interpret in the past, in at least some cases apparently reflects antigen sharing or cross-reactivity. This conclusion is based on finding some clones of each PLT that are restimulated by only cells of one individual (with respect to those tested) and other clones from the same PLT that are restimulated by cells of that individual and by cells of another

Table 3 Proliferative response of non-cloned PLT versus limiting dilution 'cloned' alloactivated cells

Responding cells	Restimulating cells			
	A _x	B _x	C _x	D _x
Original PLT	173	5,152	831	207
'clones'				
40-10E	543	6,000	536	375
40-7E	641	44,681	906	489
40-8E	576	3,842	2,313	710
40-4E	814	4,634	2,475	683
40-9D	881	999	993	742

'Clones' were obtained from limiting dilution of day 4 MLC blast cells and were grown in Terasaki microtitre wells in the presence of feeder layers and TCGF. The cells were derived from wells which received on average 40 cells per well at the time of dilution into Terasaki plates. 40-9D is an example of a clone giving no proliferative response.

individual. As both HLA-D and DR antigens can restimulate PLT cells^{9,10} it is most likely that cloning will allow fine structure dissection and analysis of the complex of HLA-D region-encoded antigens. PLT responses can be obtained with as few as 5×10^3 responding cells (ref. 11 and unpublished data); thus, cloning makes reagents available that could be used to type more than 1×10^4 individuals per clone. Although our emphasis has been on the testing of PLT reactive clones, the fact that cytotoxic T lymphocyte clones can also be obtained in these conditions, now makes possible certain experiments in man for the study of T lymphocyte function using homogeneous cell populations.

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Epidermal growth factor receptors increase during the differentiation of embryonal carcinoma cells

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Mouse teratocarcinoma stem cells (embryonal carcinoma, or EC cells) bind very small amounts of mouse epidermal growth factor (EGF) and the latter hormone seems to have no stimulatory effect on the growth of two cloned lines of EC cells. However, when EC cells are induced to differentiate into large flat endoderm-like cells (END cells), EGF receptors increase in number reaching a plateau in 6 to 8 days. At 8 to 10 days after induction, END cells multiply very slowly, but when EGF is added (3×10^{-10} M) to the medium, cell division is stimulated and a further change in morphology occurs. This letter describes the binding characteristics and numbers of the EGF receptors on EC and END cells and shows that exogenous retinoic acid increases the numbers of EGF receptors on END cells. We were unable to find endogenous competing factors produced by EC cells. Such factors could account for the lack of detectable binding of EGF on these cells. As EC cells differentiate to END cells, so the ability of the cells to form tumours is reduced^{1,2}. Since this change is accompanied by an increase in the number of EGF receptors there may be a relationship between these two events.

The experiments were performed on the cloned cell lines PC13 clone 5 (ref. 3) and OC15S1 (ref. 4) which were both derived from the transplantable 129/JSV strain teratocarcinoma OTT6050 (ref. 5). Both clones are pluripotent; OC15S1 differentiates in culture to form muscle and nerve which express biochemical markers characteristic of these cell types^{6,7}, while

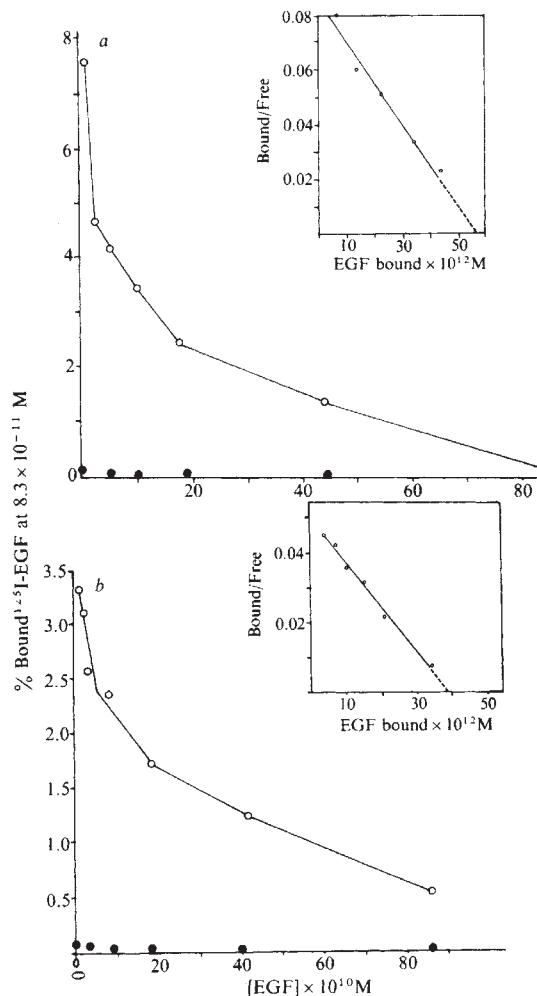


Fig. 1 Binding of ^{125}I -EGF to monolayers derived from *a*, PC13 clone 5 and *b*, OC15S1. EC cells were plated at 1×10^5 cells per 50-mm gelatinised dish in modified α -medium containing 10% fetal calf serum and 3×10^{-6} M retinoic acid¹⁰. Over 7 days with one change of medium, 1×10^6 END cells formed. Duplicate dishes at each point were used in binding assays. OC15 END cells were formed both as above and by the low-density plating method which does not require retinoic acid^{9,10}. ^{125}I -EGF was prepared according to the following protocol: 4 μg EGF in 4 μl 0.3 M sodium phosphate buffer at pH 7.5 was added to 10 μl Na ^{125}I (Radiochemical Centre, 100 mCi ml $^{-1}$). Chloramine-T 5 μl (2.5 mg ml $^{-1}$ in water) was then added and the mixture agitated for 35–40 s after which 5 μl sodium metabisulphite (5 mg ml $^{-1}$ in 0.3 M sodium phosphate buffer) and 100 μl bovine serum albumin (25 mg ml $^{-1}$ in phosphate buffer) were added in quick succession. The ^{125}I -EGF was purified on a column (60 cm by 1.2 cm diameter) of Sephadex G-50 in phosphate-buffered saline containing bovine serum albumin at 1 mg ml $^{-1}$. Three peaks were obtained: the first contained damaged and over-iodinated material and the last free iodine. The middle peak (^{125}I -EGF) was split into 50- μl aliquots and frozen at -20°C . The binding assay buffer contained Earle's balanced salt solution, HEPES (5 mM), and bovine serum albumin (1 mg ml $^{-1}$). Cell monolayers were washed twice with 2 ml buffer and then reacted with ^{125}I -EGF, finally 8.3×10^{-11} M, (970 Ci mmol $^{-1}$ 250,000 c.p.m.) and with varying amounts of unlabelled EGF (from zero to 4×10^{-8} M) in a final volume of 2 ml buffer. The dishes were incubated at room temperature ($\sim 22^\circ\text{C}$) for 90 min with intermittent shaking. The mixture was aspirated and the dishes were washed three times with ice-cold binding buffer. Cells were removed with 0.5 M NaOH and counted in a Hewlett-Packard γ -counter. The data points shown are the means of at least three separate determinations and values are normalised to 10^6 cells. Nonspecific binding was determined by measuring the bound counts in the presence of a large excess (520-fold over ^{125}I -EGF) of unlabelled EGF and was generally less than 5% of the specifically bound counts. ●, EC cells; ○, END cells. Inserts, Scatchard plots used to calculate binding constants.

Table 1 Comparison of binding of ^{125}I -labelled EGF to various cell lines

Cell type	Additions	% EGF bound at 10^{-10} M per 10^6 cells	% EGF bound relative to PC13 END
PC13 EC	None	<0.1	<1.4
PC13 END	RA (1 d)	1.3	17.6
	RA (2 d)	3.2	43.2
	RA (5 d)	4.9	66.2
	RA (6 d)	7.2	97.3
	RA (8 d)	7.4	100.0
OC15 EC	None	<0.05	0.7
OC15 EC	RA (1 d)	0.15	2.0
OC15 END	None (7 d)	1.08	14.5
	RA* (7 d)	3.31	44.7
PSA5E	None	1.3	17.6
PSA5E	RA (2 d)	1.25	16.9
PYS-2	None	<0.1	<1.4
PYS-2	RA (2, 4, 6 d)	<0.1	<1.4

The conditions for binding were as detailed in Fig. 1 legend. When retinoic acid was used it was added at 3×10^{-5} M in dimethyl sulphoxide (DMSO) to a final concentration of 3×10^{-6} M. As far as possible, cell densities were maintained at 10^6 cells per 50-mm dish. Separate experiments showed that the amount of ^{125}I -EGF bound increased linearly with cell density and that there was no decrease in binding as confluency was approached. The possibility that retinoic acid competed with ^{125}I -EGF for receptor sites was eliminated in separate control experiments. Addition of DMSO alone to EC cells affected neither the morphology of the cells nor the amount of ^{125}I -EGF bound.

* Retinoic acid was removed for 24 h before the assay.

PC13 forms these and other cell types when injected into adult hosts⁸. Both clones form large flat cells when grown in retinoic acid (3×10^{-6} M); and similar END cells are formed when OC15S1 cells are plated at low density and grown in a minimal medium^{9,10}. Retinoic acid has been shown previously to induce EC cells to develop into endoderm-like cells which secrete plasminogen activator^{11,12}, type I-like collagen¹⁰ and basement membrane protein¹³.

EGF is a potent polypeptide mitogen extracted from mouse submaxillary glands (EGF, Collaborative Research). It interacts with specific cell-surface receptors on mouse and human fibroblasts, glial cells and corneal epithelial cells^{14,15}. When mature END cells were incubated with ^{125}I -labelled EGF at 8.3×10^{-11} M in the presence of increasing concentrations of unlabelled EGF, specifically bound EGF was detected (Fig. 1). The data shown in Fig. 1 were used to estimate the binding association constants (K_a) and the numbers of EGF receptors per cell by Scatchard plots¹⁶. The value of $1.4 \times 10^9 \text{ M}^{-1}$ and $1.2 \times 10^9 \text{ M}^{-1}$ for PC13 END and OC15 END respectively are close to the K_a of $2.3 \times 10^9 \text{ M}^{-1}$ for human foreskin fibroblasts obtained by Carpenter and Cohen¹. The latter cells have 10^5 EGF receptors per cell whereas END cells have slightly fewer: 7×10^4 for PC13 END and 3.1×10^4 for OC15 END. The number of EGF receptors in a mouse fibroblast line (STO¹⁷) was found to be similar to that in human fibroblasts; STO was therefore used as a positive control in these experiments.

The formation of PC13 END from EC cells takes place gradually on exposure to $3\text{--}6 \times 10^{-6}$ M retinoic acid, and the original small round EC cells form extremely large and flat cells (Fig. 2a, b, c). During the development of END cells there is some cell death¹ and this raises the possibility that during the formation of END cells, a minor subpopulation of EC cells with EGF receptors survives and overgrows during the END cell transition. However, this phenomenon is unlikely to explain our results with PC13 END cells because 24 h after the addition of retinoic acid, cell numbers had less than doubled while the amount of EGF bound had increased 12-fold. Table 1 shows that there is a gradual increase in the amount of EGF bound per cell during the transition; this is probably due to the gradual formation and maturation of END cells. PC13 EC and OC15S1 EC cells bound very little ^{125}I -EGF. It is clear from Table 1 that

OC15 END cells which were formed in the presence of retinoic acid were able to bind higher levels of ^{125}I -EGF than similar END cells formed in the absence of exogenous retinoic acid. We also tested two teratocarcinoma-derived differentiated cell lines which were known to have endoderm-like properties. PSA5E cells¹⁸ bound ^{125}I -EGF and binding was unchanged by the addition of retinoic acid; PYS-2 cells¹⁹ did not bind significant amounts either in the presence or absence of retinoic acid. These cell lines are thought to possess endoderm properties similar to those of visceral yolk sac endoderm and parietal endoderm, respectively. This finding raised the possibility of differential distributions of EGF receptors in embryos and preliminary studies confirm this (manuscript in preparation).

Retinoic acid is required for the transition of PC13 EC into END cells, and promotes the formation of larger numbers of EGF receptors on OC15 END (Table 1). In contrast, a third effect occurs on removal of retinoic acid from PC13 (and OC15) END cells for various times before applying the ^{125}I -EGF binding assay. The number of EGF receptors on PC13 END cells was found to have increased to 28% above normal after 2 days culture in the absence of retinoic acid. Opposing effects of retinoic acid have also been documented in respect of its ability both to enhance tumour growth and to oppose promoters of tumour growth (see ref. 20 for review).

Figure 3 shows the effect of EGF on the growth of PC13 END cultured in 50-mm dishes seeded at 1×10^5 cells per dish. In one series EGF was absent and the cell number either decreased or remained the same; this depended on the age of the cells and the density of seeding but the continued presence of retinoic acid had no effect. In the other series, the addition of EGF at 2 ng ml^{-1} caused cell numbers to increase three- to fourfold in 6 days. The continued presence of retinoic acid appeared to be necessary to maintain this rate of cell division. OC15S1 END cells responded similarly to EGF (not shown). After about 2

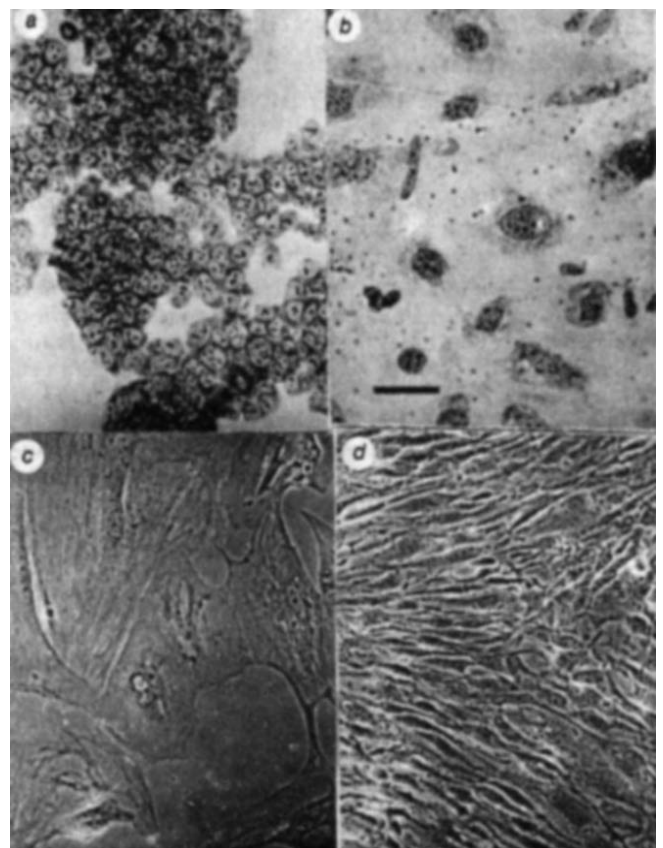


Fig. 2 Photomicrographs of; a EC cells; b, END cells stained with Giemsa; c, END cells; d, END cells after 4 days growth in EGF (3.7×10^{-10} M), phase contrast. All at the same magnification, scale bar, 50 μm .

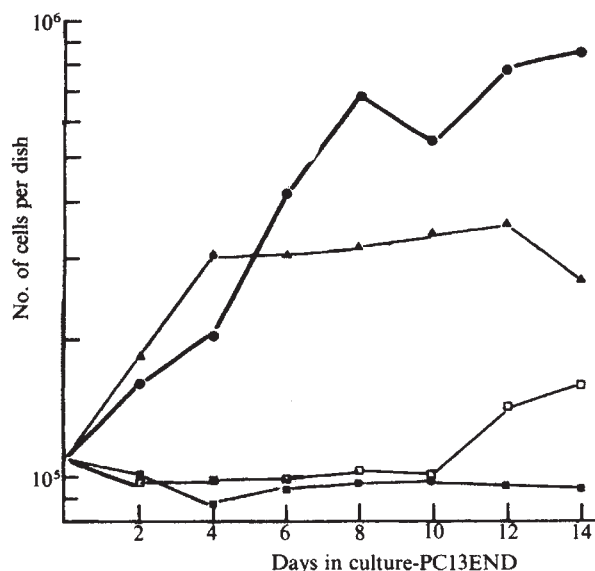


Fig. 3 Growth curves of PC13 END cells. END cells were formed as described in Fig. 1 legend and then reseeded at about 1×10^5 cells per 50-mm dish and cultured for two more days in medium containing 3×10^{-6} M retinoic acid. Medium was replaced with one containing 3.7×10^{-10} M EGF (▲); 3.7×10^{-10} M EGF and 3×10^{-6} M retinoic acid (●); with retinoic acid (■); or with neither (□). Cells in duplicate dishes were counted every other day and the medium was changed every 3 days. Similar results were obtained in each of three experiments with PC13 END and twice for OC15 END cells.

days in contact with EGF, PC13 END cells changed to a fibroblastic appearance (Fig. 2d) and later when more dense, they formed multilayers.

In a similar experiment the effect of EGF on the growth of OC15S1 and PC13 EC cells was tested (not shown). No effect was detected on the already fast multiplication rate of these cells (approximate doubling time 17 h). This finding was in accord with the very low binding of 125 I-EGF to EC cells shown in Table 1. It was possible that EGF receptors were present on EC cells but were masked by endogenous growth factors. Such factors have been described²¹: they were secreted by Moloney sarcoma virus (MSV)-transformed mouse cells; they had a mitogenic action on rat fibroblasts; and they competed with 125 I-EGF in the EGF receptor-binding assay. We have been unable to find any endogenous growth factors secreted into the medium or in the cytosol of PC13EC cells, but END cells shed a material which may contain EGF receptors since it inhibited the binding of 125 I-EGF to PC13 END cells by nearly 90%. During the incubation, END cells remained healthy and cell breakdown could not account for any release of proteases which might have affected the binding assay. It is also known that human lymphocytes shed insulin receptors when cultured in serum-free medium²².

The main finding of these experiments is that both PC13 and OC15S1 EC cells, when allowed to transit into END cells, develop EGF receptors while also changing in morphology and in macromolecular secretory products¹. We believe that the transition is a differentiative process and that an endoderm-like cell is the product¹⁰⁻¹³. END cells have a very low incidence of tumour formation: in contrast, EC cells are highly tumorigenic in adult hosts and therefore lack the growth controls which operate on normal cells. Hence the very low numbers of EGF receptors on EC cells and the very large numbers on END cells may indicate that EGF receptors are part of the growth control of these cells. Cells which are transformed by MSV²³ or by phorbol esters²⁴, have a decreased number of available EGF receptors (or reduced-affinity receptors²⁵) and transformation can be blocked by retinoids²⁶. It is possible that the blocking effect of retinoids is mediated through the renewed operation of

EGF and the EGF-receptor system, since we show here that retinoic acid stimulates the production of EGF receptors. The experiments reported above demonstrate an increase in the numbers of EGF receptors with differentiation and the results are consistent with the hypothesis that teratocarcinoma tumour stem cells (and early embryonic ectoderm therefore) only become subject to growth control by EGF after their differentiation into cells of a more specialised and restricted type. It should be possible to test this hypothesis further.

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Sequential X chromosome inactivation coupled with cellular differentiation in early mouse embryos

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Only one of the two X chromosomes is active in each somatic cell of adult female eutherian mammals, making the females (XX) equivalent to the males (XY) with respect to X chromosome dosage¹⁻⁴. Biochemical analyses showing that both X chromosomes are active in female mouse embryos in mid-cleavage stage⁴⁻⁸ indicate that X chromosome differentiation involves inactivation. This event occurs in most or all cells of the embryo at the blastocyst stage^{4,7-9}, when there are two cell types, the outer sphere of trophectoderm cells and the inner cell mass (ICM). Because there is genetic evidence that both X chromosomes are potentially active in ICM cells¹⁰, it has been suggested that X chromosome inactivation has occurred in only the trophectoderm cells⁹. Further, one of us (M.M.)⁴ has proposed that X chromosome differentiation is linked to cellular differentiation, occurring at different times in different cell populations as they 'depart' or terminally differentiate from a pluripotent fetal 'stem line' (Fig. 1). Analysis of a large number of inner cell masses isolated immunosurgically from female blastocysts has yielded data consistent with the presence of two active X chromosomes¹¹, but ICMs are so small that the biochemical assay used was at the limit of its accuracy. (Neverthe-

less, a computer analysis of the data⁸ indicated two ICM populations differing twofold with respect to X chromosome activity.) More tissue is available for analysis in post-implantation embryos, in which, on the above hypothesis, we would expect two active X chromosomes in the pluripotent epiblast region before gastrulation, but only one in the corresponding extra-embryonic ectoderm (a trophoctoderm-derived tissue¹²) and primary endoderm (ICM-derived¹², see Fig. 1). We report here that this is the case; we also show that inactivation is complete in the epiblast (fetal precursor) cells between 6.0 and 6.5 d of gestation at the onset of gastrulation.

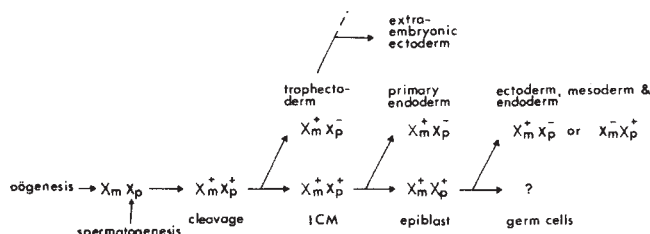


Fig. 1 Hypothesis linking X chromosome differentiation to cellular differentiation⁴. X chromosome differentiation is shown as occurring in different cell lineages as they differentiate (m, maternal; p, paternal; +, active; -, inactive). Tissues differentiating early, that is, trophoctoderm and primary endoderm, show preferential inactivation of the paternally inherited X chromosome¹⁶⁻¹⁸, suggesting some memory mechanism² detecting the parental origin of the two X chromosomes. This memory mechanism is presumably lost by the time of gastrulation when inactivation is random.

We used a biochemical assay to measure simultaneously the activities of two enzymes in dissected tissues of individual post-implantation embryos. These enzymes are the X-coded hypoxanthine phosphoribosyl transferase (HPRT, EC 2.4.2.8) and the autosomally coded¹³ adenine phosphoribosyl transferase (APRT, EC 2.4.2.7). The assay has been previously described⁶ (see Fig. 2 legend) and its validity recently unequivocally confirmed by thin layer chromatography (M.I.H. and M.M., unpublished). The results are expressed as a ratio of HPRT to APRT activities for a particular dissected tissue from an individual conceptus, thus eliminating sampling errors which would be introduced by variation in the amount of pure dissected tissues recovered. We expect the ratio of X-coded HPRT to autosomally coded APRT activity to be twice as high in an XX tissue as in an XY tissue if both X chromosomes are active in that particular region of the female conceptus.

Swiss female mice (MF1, Olac UK) were mated between 1100 and 1700 h to MF1 males in a reversed day/night room. The embryos were isolated from the uteri of these females 6 days later (6-d conceptuses), the trophoctoderm and ectoplacental cone discarded and the egg cylinders dissected into three tissue populations: epiblast, extra-embryonic ectoderm and primary endoderm (see Fig. 2a). Extracts of these tissues were assayed for HPRT and APRT activity, and the ratios of activities of these enzymes per tissue per embryo are given in Fig. 2. It can be seen that whereas the values for the extra-embryonic ectoderm and primary endoderm regions (Fig. 2c, d) show unimodal distributions with a less than twofold spread, the values for the epiblast region (Fig. 2b) are bimodal. A computer analysis indicated two populations of epiblasts in the proportion 0.6:0.4 (lower: higher) and gave estimates compatible with a twofold difference between the means. By analogy with previous work^{4,6,11}, we assume that the bimodality is due to a bimodal distribution of X-coded HPRT activities. The results shown in Fig. 2 suggest that X chromosome inactivation has already

occurred in extra-embryonic ectoderm and primary endoderm, whereas there seem to be two populations with respect to X chromosome activity in epiblast regions.

Several of these epiblasts were sexed by karyotyping their respective extra-embryonic regions. The results are shown in Fig. 3. The mean of HPRT/APRT ratios for the female epiblasts is significantly higher than that for male epiblasts (Student's *t*-test gives $t_{24} = 3.14$, $P < 0.01$). The fact that more epiblasts in Fig. 2 are located in the lower peak (see above) is consistent with the overlap between male and female values indicated in Fig. 3, and suggests that X chromosome inactivation has already occurred in about one-fifth of the XX epiblasts. One possible explanation for the bimodality in the epiblasts is that X chromosome inactivation has occurred at the same time as in primary endoderm, but that HPRT activity observed in 6-d epiblasts is due to enzyme which was present in the cells before this event. We consider this explanation unlikely because the 6-d epiblast is the most rapidly proliferating region of the embryo¹⁴. We do not know the spread in timing of the inactivation event in individual epiblast cells, but we can conclude that it occurs at around 6 days' gestation.

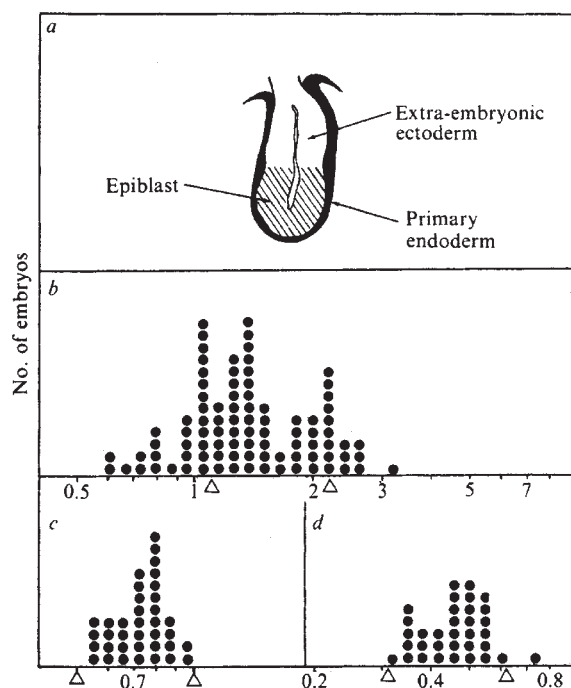


Fig. 2 Ratios of HPRT to APRT activities in dissected tissues of 6-d egg cylinders. a, Diagrammatic representation of 6-d egg cylinder; b, HPRT:APRT in individual epiblasts; c, HPRT:APRT in individual extra-embryonic ectoderm regions; d, HPRT:APRT in individual primary endoderm regions. The egg cylinders were isolated from decidua, washed in PB1 medium²⁴ containing 0.4% polyvinylpyrrolidone instead of albumin, and transferred to PB1 containing 0.5% trypsin and 2.5% pancreatin for 10 min at 4 °C. Enzyme treatment was terminated by the addition of fetal calf serum (to 2%). The egg cylinders were washed in PB1, the three components of each separated using finely drawn Pasteur pipettes and glass needles and transferred separately in 5 μ l of medium to 10- μ l microcaps. The microcaps were sealed and freeze-thawed three times in liquid nitrogen to lyse the cells. After centrifugation, the supernatants were transferred to 50 μ l of reaction mix containing sodium phosphate buffer (35 mM, pH 7.4), magnesium chloride (5 mM), phosphoribosyl pyrophosphate (2 mM), ³H-guanine sulphate (10 μ M, specific activity 610 Ci mol⁻¹) and ¹⁴C-adenine (10 μ M, specific activity 286 Ci mol⁻¹). Reactions were carried out at 37 °C for 2 h. Further details are given in ref. 6. Arrowheads indicate a twofold span on the horizontal axis (logarithmic) for each distribution.

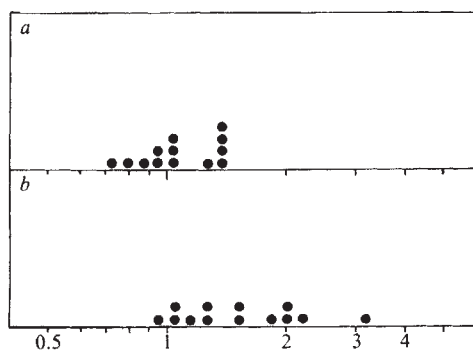


Fig. 3 Ratio of HPRT to APRT activities on 6-d male (a) and female (b) epiblasts. Embryo isolation and dissection, and enzyme assays were carried out as described in Fig. 2 legend. Individual extra-embryonic ectoderms were karyotyped as follows: tissues were incubated individually in droplets of Dulbecco's modified Eagle's medium (DMEM) containing 5% fetal calf serum and incubated overnight at 37 °C in a 5% CO₂-in-air atmosphere. They were transferred to the same medium containing 0.1 µg ml⁻¹ colcemid and incubated for 4 h, followed by transfer to 1% sodium citrate for 45 min at room temperature, then to fresh fixative (3:1 ethanol/glacial acetic acid) at 4 °C for a minimum of 30 min. Spreads were made by transferring the tissue in a microdrop of fixative to a clean slide, promptly adding two droplets of 60% acetic acid and spreading on a hot plate at 60 °C. The preparations were stained in Giemsa diluted 1:10 in a phosphate buffer, pH 6.9, for 10 min. Sex determination was based on the presence of two versus three small chromosomes.

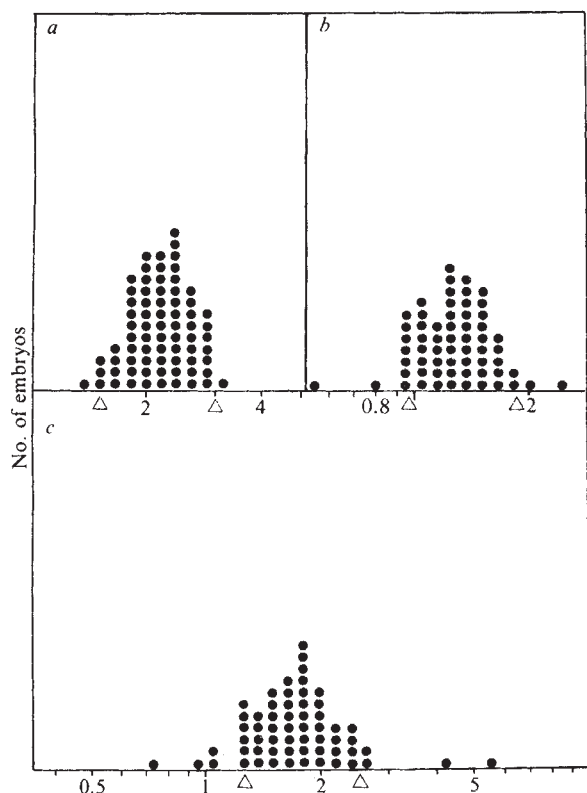


Fig. 4 Ratios of HPRT to APRT activities in dissected tissues of 6½-d egg cylinders. a, HPRT:APRT in individual epiblasts; b, HPRT:APRT in individual extra-embryonic ectoderm regions; c, HPRT:APRT in epiblast/HPRT:APRT in extra-embryonic ectoderm, per individual egg cylinder. Embryo isolation and dissection, and enzyme assays were carried out as described in Fig. 2 legend.

Twelve hours later in development there is no difference between the distributions of HPRT/APRT ratios in epiblasts and the other tissues (Fig. 4). Here, dissected epiblast and extra-embryonic ectoderm regions of 6½-d conceptuses (6½ d following mating in a normal day/night mouse room) show similar unimodal distributions which are incompatible with two populations with twofold differences in X-coded HPRT activity. The ratios of the HPRT/APRT values in epiblast to those of the corresponding extra-embryonic ectoderm are also convincingly unimodal (Fig. 4c). We conclude that X chromosome inactivation has occurred in epiblast regions by 6½ days' gestation. Kozak and Quinn¹⁵ analysed individual post-implantation embryos for the X-coded phosphoglycerate kinase (PGK, EC 5.3.1.1). They failed to detect a bimodal distribution of (presumably) whole egg cylinders (epiblast+extra-embryonic ectoderm+endoderm), but the embryos they analysed seem to have been more closely equivalent to our 6½-d embryos.

Our findings provide strong evidence for the hypothesis outlined in Fig. 1 that X chromosome differentiation in early development is linked to cellular differentiation. The activity status of the X chromosomes in the germ cell line is not clear. It is of interest that tissues arising from trophectoderm and primary endoderm isolated from later post-implantation stages show preferential inactivity of the paternally inherited X chromosome¹⁶⁻¹⁸. Cells differentiating early in development seem to have some memory mechanism which recognises the origins of the two X chromosomes.

The coupling of X chromosome differentiation with cellular differentiation may mean that the mechanisms involved in 'switching-off' the whole X chromosome are similar to those which select activity or inactivity in different autosomal regions characteristic of specialised cell function. Ohno¹⁹ has suggested that similar basic mechanisms might be expected for X-inactivation and cellular differentiation. However, attempts to examine X-inactivation by reversing the process have yielded little information; apart from localised derepression²⁰, strong selective pressures have failed to reverse the inactive state of the silent X chromosome^{21,22}. Martin *et al.*²³ have shown that X chromosome inactivation occurs with *in vitro* differentiation of an XX teratocarcinoma cell line. One of the implications of their findings, and ours, is that two active X chromosomes may be incompatible with the differentiated state.

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Zoological origin of gonadotropin subunits and association kinetics

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Mammalian luteinising hormone (LH) is an association of two dissimilar subunits, α and β . *In vitro* studies, mainly using difference spectrophotometry, had shown that this phenomenon was slow, especially at low temperatures. If the situation was the same in poikilotherms, it would probably make gonadotropin (GTH) synthesis difficult for these animals in a cold environment. We have found that the formation of a teleost gonadotropin is in fact strikingly more rapid and less thermodependent than formation of mammalian LH. Also, studies with a fish-mammal hybrid molecule have allowed us to estimate the respective influence of the α and β subunits in determining these differences.

Association of mammalian LH subunits is a very slow process. Various methods of study have given convergent results indicating that maximal association is not attained before several hours at 37 °C, or several days at 20 °C¹⁻³. Indeed the phenomenon is highly thermodependent^{3,4}; at 0 °C no significant association was observed after 6 hours³.

It became possible to investigate this problem in a poikilotherm when α and β subunits of GTH from a teleost fish, the carp (*Cyprinus carpio* L.) were isolated, and shown to reassociate⁵. Carp GTH(c-GTH) and its subunits were prepared according to Burzawa-Gerard⁵ and dissolved in 0.02 M phosphate buffer pH 7.4 containing 0.1 M KCl. Carp GTH α and β were mixed in equimolar amounts assuming respective molecular weights of 14,000 and 17,000⁶. Subunit association was followed by difference spectrophotometry in conditions as used previously for the study of ovine LH (o-LH) formation^{2,7} at three concentrations of each subunit (1.9, 3.6 and 6.8 μ M, recalculated from absorbance at 280 nm). At 20 °C and for a concentration of 6.8 μ M, the percentage association was 75% after only 45 min of incubation and was still clearly increasing at this time⁸. This indicated that virtually all the subunits were able to associate, and this was confirmed by bioassay; indeed a 60–100% recovery of biological activity (measured by the frog spermiation test) was obtained, depending on the conditions of incubation⁹.

Figure 1 shows the results obtained at 4 °C. When the inverse of the concentration in free subunits was plotted as a function of

time, parallel straight lines were obtained with the three different initial concentrations of subunits, indicating an apparent second order reaction with a rate constant (k) equal to $78 \pm 9 \text{ M}^{-1} \text{ s}^{-1}$. Confirming this, a first order plot was nonlinear with estimated rate constants depending on subunit concentration. At 20 °C, the results (not shown) were qualitatively similar to those at 4 °C but k was $149 \pm 24 \text{ M}^{-1} \text{ s}^{-1}$. Between 4 and 20 °C the Q_{10} of the reaction was 1.5 (limits for $P = 0.05$: 1.44–1.57). These values are strikingly different from those measured for o-LH (k 20 °C = $0.86 \text{ M}^{-1} \text{ s}^{-1}$; $Q_{10} = 4.8$ between 10 and 37 °C)⁴. Indeed, even at the low temperatures at which fish tend to live, the formation of GTH from its subunits may occur at a reasonable rate. Note that the changes in the circular dichroism spectrum seen during hormone formation from subunits are very similar for c-GTH and for o-LH⁷. As for mammalian hormones, thermal perturbation spectroscopy of c-GTH showed that three tyrosine residues (3.2 ± 0.2) were shielded from the solvent when the subunits were associated. These data show that similar structural changes occur during the formation of c-GTH or mammalian LH from their subunits, although these associations proceed at markedly different rates.

An attempt was made to determine the respective roles of α and β subunits in the important differences observed between the formation kinetics of c-GTH and o-LH. To this end, we took advantage of the fact that bovine (b)-LH α and c-GTH β are able to associate and to form a hybrid molecule possessing a very high biological activity on frog spermiation¹⁰. Bovine LH α and c-GTH β were mixed in equimolar amounts and the association process was followed by difference spectrophotometry at two concentrations (3.3 and 6.3 μ M) and two temperatures (4 and 20 °C (Fig. 2)).

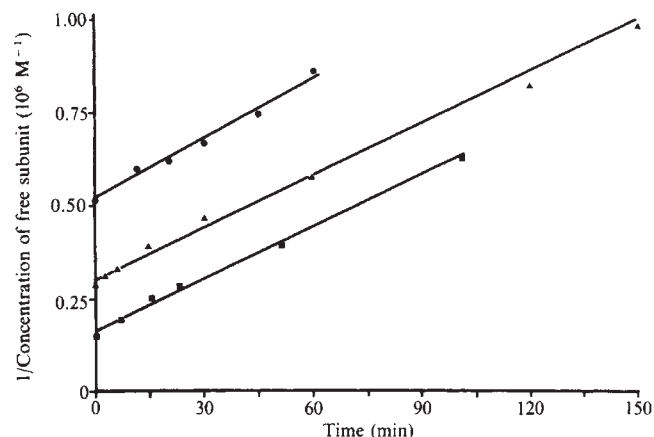


Fig. 1 Second order rate plot of the association of c-GTH subunits at 4 °C. Equal volumes (680 μ l) of α or β subunits solutions were placed into the compartments of a double-sector cell. Two such cells were prepared. After equilibration of the temperature (zero time) mixing was accomplished in one of them (sample cell), the other, unmixed, cell being used as reference. The difference in absorbance at 287 nm was scanned at various times on a Cary 118 spectrophotometer. Separately, the maximum absorbance difference (ΔA_{max}) corresponding to a 100% unfolding of c-GTH was measured between two solutions of native hormone at the same concentration (8.6 μ M), one of which was acidified at pH 2.0 with 5 M HCl (complete unfolding of c-GTH); the ratio of ΔA_{max} to the A_{280} of the native hormone solution was 0.11. The percentage of association at each time was then calculated as the ratio: ΔA_{287} between mixed and unmixed cells/ $0.11 \times A_{280}$ of the native hormone solution at the concentration under study. The concentration of free, native subunits was determined from this percentage by assuming that all the subunits present at zero time were able to associate (see text). The inverse of the free subunit concentration was plotted as a function of time. Experiments were carried out at three subunit concentrations: 1.9 μ M (●), 3.6 μ M (▲) and 6.9 μ M (■). The correlation coefficient was >0.99 in each case.

Table 1 Thermodynamic parameters for the formation of c-GTH, hybrid b-LH α -c-GTH β , and o-LH from subunit components at 20 °C

	Enthalpy (ΔH^* , kcal M^{-1})	Entropy (ΔS^* , cal $\text{K}^{-1} \text{M}^{-1}$)	Free enthalpy (ΔG^* , kcal M^{-1})
c-GTH	+5.38	-30.1	+14.2
Hybrid	+6.62	-31.3	+15.80
o-LH†	+23.5	+21.5	+17.2

The thermodynamic parameters were calculated by plotting $\ln k$ against $1/T$ (T , temperature in K). Measuring the slope of the curve ($-E/R$), where E is the energy of activation) we obtained $\Delta H^* = E - RT$, then $\Delta G^* = RT (\ln KT/h - \ln k)$ and $\Delta S^* = \Delta H^* - \Delta G^*/T$ (K , Boltzmann constant; h , Planck constant).

† Data calculated from ref. 4.

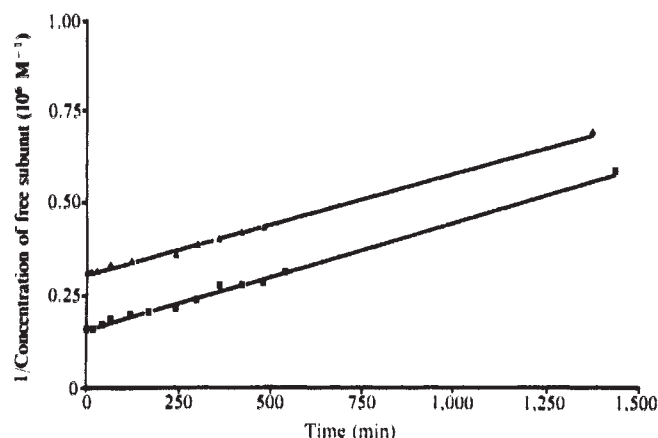


Fig. 2 Second order rate plot of the formation of hybrid b-LH α -c-GTH β from subunits at 4°C. Association was followed as described in Fig. 1. The maximum absorbance difference corresponding to a 100% unfolding was measured between two solutions of purified hybrid at 6.2 μ M, one of which being acidified at pH 2.0 with HCl 5 N; the ratio of $\Delta A_{\max}$ to A_{280} of the hybrid solution was 0.094. Purified hybrid was obtained as follows; a mixture of b-LH α and c-GTH β (124 μ M each) was incubated for 16 h at 20°C, gel-filtered on Sephadex G-100 in NH_4HCO_3 0.1 M; the fractions with an exclusion coefficient from 0.1 to 0.32, corresponding to a protein peak with a molecular weight close to 30,000, were pooled and lyophilised. Calculations were carried out as indicated in Fig. 1 legend. The inverse of the free subunit concentration was plotted as a function of time. Association kinetics were studied at two subunit concentrations: 3.3 μ M (▲) and 6.3 μ M (■). The correlation coefficient was >0.99 in each case.

At 20°C and for a concentration of 6.3 μ M, the percentage of association was about 80% after 900 min and it was still clearly increasing at this time. Bioassays confirmed that virtually all the b-LH α and c-GTH β molecules were able to associate: a mixture of these two subunits (62 μ M each) was incubated for 6 h at 25°C; then the dose inducing 50% positive responses (D50) in the frog spermiation test was 0.15 μ g (0.09–0.25) not different from the D50 for purified hybrid prepared as described in the Fig. 2 legend.

As for c-GTH subunits, the kinetics of association of b-LH α and c-GTH β indicated an apparent second order rate reaction at both 4°C (Fig. 2) and 20°C (data not shown). The k values were 4.8 $\text{M}^{-1}\text{s}^{-1}$ (5.0–4.6) at 4°C and 9.8 $\text{M}^{-1}\text{s}^{-1}$ (9.7–9.9) at 20°C; the Q_{10} within this interval was 1.5.

Thermodynamic parameters for the association reaction were calculated and are presented in Table 1. Because of the complexity of the mechanisms of association, and as shown with o-LH⁴, no straightforward interpretation of these parameters can be proposed. However, it is clear that the rate of the hybrid formation is intermediate between those for the two mother hormones whereas the activation enthalpy is very close to that for the fish hormone.

Thus differences in the structure of the α subunit do not seem to modify the influence of temperature on the association rate: the thermodependence of the reaction is determined solely by the β subunit. In contrast, not only the β subunit (as suggested from work on mammalian hormones¹¹) but also the α subunit, contribute to determine the association rate at a given temperature.

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GABA-mediated biphasic inhibitory responses in hippocampus

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γ -Aminobutyric acid (GABA) can have a biphasic effect on membrane potential when applied to certain central nervous system neurones^{1–3}. Typically with iontophoresis, this effect is a negative-positive sequence of deflections and both phases are associated with an increase in conductance. However, iontophoretic application of neurotransmitter substances can produce effects even in cases where ordinary synaptic transmitter release is impossible; for example, on dorsal root ganglion cells. Thus, it is important in assessing the physiological relevance of the depolarising GABA response to determine if similar actions are produced synaptically. In addition, although the depolarising aspect of the GABA response has been attributed to activation of dendritic receptors, this identification has remained uncertain. Here, we have used the hippocampal slice preparation to demonstrate that a biphasic inhibitory response results from orthodromic, but not antidromic electrical stimulation in the presence of anaesthetic concentrations of pentobarbital. (For the sake of brevity, we use the term 'biphasic inhibitory postsynaptic potential (i.p.s.p.)' to describe the response complex, although it seems likely that a combination of two distinct events, rather than two parts of a single event, occurs.) GABA antagonists and tetrodotoxin (TTX) were used to show that the depolarising phase is due to synaptically released GABA acting on dendritic sites in the CA1 region. Both negative and positive phases are associated with a large conductance increase, and both block cell firing, indicating their inhibitory nature. The ionic mechanism of the depolarising phase is not fully understood but seems to involve a chloride conductance increase. Although the functional significance of the depolarising sign of the i.p.s.p. is not clear, the dendritic location would provide very effective control over dendritic excitability.

The slice preparation we use has been described elsewhere⁴. The major modification adopted here is that slices are held fully submerged between two pieces of nylon mesh. This arrangement permits prolonged stable recordings and the evaluation of drug effects from a single cell in both control and drug conditions. To oxygenate the slices adequately, the temperature of the bathing medium was held at 28–30°C (ref. 5). The medium consists of 116.4 mM NaCl, 5.37 mM KCl, 2.5 mM CaCl_2 , 1.3 mM MgSO_4 , 0.92 mM Na_2PO_4 , 26.2 mM NaHCO_3 and 11 mM glucose. Forty-three cells from male Sprague-Dawley rats were selected for analysis. All impalements were maintained for at least 30 min with cell resting membrane potentials over 50 mV (mean 58.1, s.d. 5.2 mV), action potential amplitudes of 78.5 ± 11.0 mV and total neurone input impedances of 45.8 ± 17.0 M Ω . Conventional intracellular recording techniques were used.

Electrical stimulation of CA1 stratum (s.) radiatum produces an excitatory postsynaptic potential (e.p.s.p.) followed by a long hyperpolarising i.p.s.p. in CA1 pyramidal cells. If 10^{-4} M pentobarbital (a concentration within the range occurring during

anaesthesia) is added to the medium, the hyperpolarisation is prolonged and usually followed by a depolarisation (5.5 ± 3.5 mV) lasting seconds (7.9 ± 3.3 s, $n = 18$; Fig. 1a). The depolarisation is more reliably elicited with strong rather than weak (near threshold) stimuli. Passage of a series of 100-ms current pulses across the membrane shows that the entire sequence is accompanied by a drop in membrane resistance (Fig. 1b).

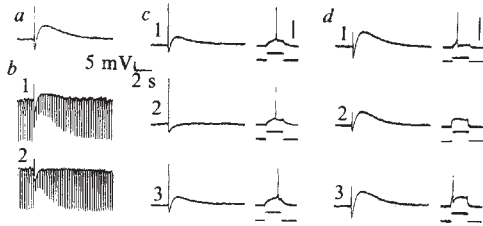


Fig. 1 Orthodromic production and dendritic localisation of depolarising i.p.s.ps in CA1 pyramidal cells. *a*, Orthodromic stimulation (s. radiatum) elicits hyperpolarisation followed by prolonged depolarisation. Resting potential (RP) -57 mV. *b*, Orthodromic and antidromic stimulation compared in a single cell. Downward deflections produced by hyperpolarising 100-ms current pulses (2 Hz) through a bridge circuit. Bridge balance was good. Reduced membrane resistance accompanies both responses; however, no depolarisation is seen with antidromic stimulation. RP, -59 mV. *c*, TTX (10^{-5} M) injected in the dendritic region abolishes depolarisation with no effect on hyperpolarisation. Action potentials produced by current pulses were unaffected immediately after TTX injection (c_2) although depolarisation was eliminated. The spike was subsequently blocked but recovered (c_3). RP, -63 mV. *d*, TTX injection in the somatic region greatly attenuates hyperpolarisation and blocks direct action potentials with little effect on depolarisation (d_2). Both hyperpolarisation and action potential recovered substantially (d_3). RP, -56 mV. All records were obtained in the presence of 10^{-4} M pentobarbital. Calibration for pen traces is 5 mV and 2 s. Calibration for oscilloscope traces is 40 mV and 1.0 nA. Current pulse duration is 100 ms in *c*, 30 ms in *d*.

GABA involvement in the depolarising phase is suggested by the observation that pentobarbital, which selectively enhances GABA action, is necessary to elicit it electrically. Further, perfusates containing GABA antagonists picrotoxin (10^{-5} M) or bicuculline (10^{-5} M) block the response ($n = 4$; data not shown) and, as seen below, iontophoretically applied GABA can mimic both hyper- and depolarising phases. These results strongly implicate GABA as the neurotransmitter for the biphasic action.

With iontophoretic pipettes independently positioned 200 μ m within the slice at mid-dendritic and somatic regions, recording from a single cell shows that, even in the absence of pentobarbital, dendritic application of GABA produces a depolarisation whereas somatic application yields a hyperpolarising-depolarising sequence (Fig. 2c), as previously shown by others². The interpretation of this pattern included the postulation of dendritic GABA receptors. Indeed, we have found that the depolarising action of GABA persists after synaptic transmission has been eliminated by changing to a low Ca^{2+} saline containing 8 mM Mg^{2+} ($n = 4$; data not shown), demonstrating that it is produced by receptors on the pyramidal cells themselves and is not an indirect effect. We hypothesised, therefore, that the electrically evoked, GABA-mediated depolarisation described above might represent a dendritic potential. To test this, we injected TTX (10^{-5} M) through a broken pipette, into the CA1 apical dendritic region. In four injections with three cells in which a large hyperpolarising-depolarising response was present, TTX selectively blocked the depolarising phase and its underlying conductance increase, but had little effect on the hyperpolarising i.p.s.p. or the action potential produced by somatic current injection (Fig. 1c). Neither resting potential nor input impedance changed and the TTX effect was reversible. The finding that the somatic spike

was not altered at a time when the depolarising phase was blocked indicates that the depolarisation requires neuronal activity in the dendritic region. In the converse experiment, TTX injection through a pipette carefully positioned near the soma resulted in a complete block of somatic action potentials and substantial reduction in stimulation-evoked hyperpolarisation, without major effect on the depolarisation ($n = 4$; Fig. 1d). (Coarse TTX injections in the somatic, but not the dendritic, regions often resulted in the simultaneous reduction of both phases which may mean the cell whose synapses produce the depolarisation exists in or near s. pyramidal.) Taken together, the results with TTX injections imply that orthodromic stimulation in s. radiatum activates dendritic synapses which mediate i.p.s.ps having a depolarising component.

Both phases of the orthodromically activated response seem to exert an inhibitory influence on the pyramidal cell as measured by their ability to block action potentials caused either by intrasomatic depolarising current pulses or by e.p.s.ps (Fig. 2a,b). Similar blocking action is seen following iontophoresis of GABA on the dendrites (Fig. 2c₂,c₃).

It was often possible to see in a single cell a biphasic response to orthodromic (s. radiatum) stimulation and hyperpolarisation of shorter duration to antidromic (alveus) stimulation, the latter closely resembling the response to orthodromic stimulation following TTX application to the dendrites (compare Fig. 1b₂ and c₂). With very high intensity stimulation, small depolarisations could occasionally be elicited with antidromic stimuli, but current spread to known orthodromic fibres in nearby s. oriens⁶ could not be ruled out in these cases. These findings indicate that

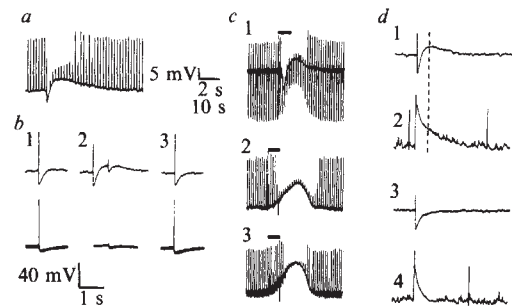


Fig. 2 Inhibitory effectiveness and chloride dependence of depolarising i.p.s.p. *a*, A train (2 Hz) of depolarising current pulses initiated full-sized somatic action potentials (amplitudes not accurately reproduced by pen recorder) which were blocked during most of the stimulation-evoked response. *b*, Inhibition of spikes elicited by just supra-threshold e.p.s.p. during a depolarising i.p.s.p. (*b*₂). Unconditioned control responses shown before and after inhibition (*b*₁ and *b*₃). Same cell as in *a*. RP, -59 mV. *c*, Effects of iontophoretic GABA (1 M) (horizontal bar) in normal medium without pentobarbital. *c*₁, Application through pipette in s. pyramidal. 100-ms current pulses given at 1 Hz (see Fig. 1b₁). RP, -55 mV. *c*₂, *c*₃, GABA iontophoresed in s. radiatum region of another cell. During depolarisation, both directly and synaptically activated action potentials are blocked (*c*₂ and *c*₃, respectively). RP, -61 mV. Iontophoretic pipettes were lowered independently 200 μ m into the slice before impaling a cell. Ejection currents were 500 nA for 5 s. *d*, Two cells from the same slice in pentobarbital. *d*₁, Recorded with a 2 M potassium methylsulphate-filled pipette; *d*₂, with a 3 M KCl pipette. Note large amplitude and prolonged time course of depolarising response in *d*₂ after Cl^- leakage into the cell. Small reversed i.p.s.ps visible on the baseline of the KCl-filled cell are capable of triggering action potentials. Antidromic responses from the same cells are shown for comparison (*d*₃, *d*₄). RP, -59 mV (*d*₁, *d*₃); -56 mV (*d*₂, *d*₄). Calibration for pen traces in *a*, *b* and *d* is 5 mV, 2 s; in *c* it is 5 mV, 10 s.

orthodromic stimulation, in addition to activating the well documented recurrent inhibitory pathway⁷, also activates a distinct set of inhibitory neurones which synapse on the dendrites of pyramidal cells and can have a depolarising action. We assume that pentobarbital merely emphasises a response which is normally present. That this drug is required to observe the depolarising i.p.s.p. may be due to the marked prolongation of GABA action which would result in a partial temporal

separation of the somatic and dendritic components. Assessment of the physiological role of the depolarisation will require more information on this point. The depolarising dendritic action of GABA in the absence of pentobarbital strengthens the notion that the dendritic i.p.s.p. normally has a depolarising component. A depolarising i.p.s.p., which is markedly enhanced by pentobarbital, has been reported to occur in olfactory cortical neurones⁸.

The ionic mechanisms of the biphasic i.p.s.p. are not entirely understood. However, elevation of intracellular chloride by leakage from a 3 M KCl electrode reverses the hyperpolarisation and increases the positive phase of the i.p.s.p. ($n = 4$; Fig. 2d). This finding, in addition to the large conductance increase, suggests that chloride is at least partially involved in both phases. To account for the depolarising sign of the dendritic i.p.s.p., it must be assumed either that the chloride equilibrium potential is positive relative to the dendritic membrane potential, or that the conductance increase also involves other ions such as sodium or calcium. These cations alone could not be responsible for the depolarisation as excitatory, perhaps regenerative, activity would be expected to occur with a large increase in their conductances. Chloride plus either sodium or calcium currents could easily produce the observed phenomena. (Experiments like that of Fig. 2b imply that the depolarisation cannot be due simply to a depolarising shift in the local i.p.s.p. equilibrium potential, as in that case the superimposed test i.p.s.p. (Fig. 2b₂) would not be hyperpolarising.) Interestingly, Dudel has recently reported⁹ that the inhibitory postsynaptic currents in crayfish muscle have a fast chloride conductance phase followed by a slower conductance phase which may involve an appreciable contribution of cations.

We have confirmed the presence of GABA-mediated dendritic i.p.s.p.s and presented evidence suggesting these form an inhibitory system distinct from the classical recurrent pathway. We have demonstrated further that there is a depolarising aspect to the synaptically activated potential in pentobarbital which seems peculiar to the dendritic region. Differences in experimental species, temperature and recording conditions may all contribute to the fact that previous investigations *in vivo* did not report the depolarising component.

Although it seems clear that selective orthodromic activation of a dendritic i.p.s.p. would provide optimal control over local dendritic events¹⁰, the functional significance of the depolarising phase is unknown. It is apparent that the pronounced conductance increase accompanying this depolarisation can predominate in inhibiting action potential generation. It remains possible, however, that the depolarisation could act to reduce the inhibitory effectiveness of the conductance increase.

The inhibitory neurones involved in the dendritic i.p.s.p. pathway have not been identified, although few cell bodies are present in s. radiatum. Possible candidates are the non-pyramidal cells which have somata near s. pyramidale and vertically orientated axons branching in s. radiatum¹¹⁻¹⁴.

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1 α ,25-Dihydroxyvitamin D₃ and 24,25-dihydroxyvitamin D₃ *in vitro* synthesis by human decidua and placenta

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The biologically active metabolite of vitamin D, 1 α ,25-dihydroxyvitamin D (1 α ,25(OH)₂D), is synthesised by successive hydroxylation of vitamin D in the liver and kidney¹. The latter organ is the only known site of 1 α -hydroxylation of 25-hydroxyvitamin D (25-OH-D)² and is also a major site of production of 24,25-dihydroxyvitamin D (24,25(OH)₂D), a metabolite involved in bone ossification³. However, 24-hydroxylation of 25-OH-D may also occur in rat intestinal homogenates and in cartilage cells^{4,5}. Accumulating data show that, during pregnancy, 1 α ,25(OH)₂D increases in the maternal circulation⁶ but is absent or present only in small amounts in the fetus^{7,8}. In comparison, 24,25(OH)₂D accumulates in fetal tissues, particularly in the skeleton⁸. Little is known, however, about the independent metabolism of vitamin D in the fetoplacental unit. It has been reported⁹ that the nephrectomised pregnant rat can synthesise 1 α ,25(OH)₂D₃ and 24,25(OH)₂D₃ and that the fetoplacental unit is the most likely site of production of such metabolites. Those data suggested that there may be independent vitamin D metabolism in the fetoplacental unit. We now report *in vitro* synthesis of 24,25(OH)₂D₃ by human placenta and of 1 α ,25(OH)₂D₃ and 24,25(OH)₂D₃ by human decidua. The human decidua is a development of the endometrium that is unique to pregnancy, originating from both epithelial and stromal cells and intimately attached to fetal structures, that is, the placenta and chorion.

Human placental, decidua and amniotic tissues from uncomplicated, full-term and from 8–12-week pregnancies were obtained within minutes of delivery or legal abortion and prepared for culture as described previously^{10,11}. Explants of the tissues were cultured with RPMI 1640 medium (GIBCO) for 48 h and then incubated with tritiated 25-OH-D₃ for 24 h as described in Fig. 1 legend. At 72 h, the tissues and medium were extracted with chloroform-methanol¹². The radiolabelled metabolites of vitamin D₃ in the lipid extracts were separated on columns of Sephadex LH-20 (Pharmacia), calibrated with authentic tritiated metabolites and developed with chloroform-petroleum ether (65:35, v/v) (Fig. 1).

Cultures of both full-term and young placental tissue synthesised 24,25(OH)₂D₃. The mean ($\pm$ s.d.) percentage of tritium recovered from the Sephadex LH-20 columns as ³H-24,25(OH)₂D₃ was 6.97 ± 0.18 (full-term placenta) and 6.82 ± 1.23 (young placenta). Cultures of both full-term and young decidua tissue synthesised 1 α ,25(OH)₂D₃ and 24,25(OH)₂D₃. The mean ($\pm$ s.d.) percentage of tritium recovered from the columns as ³H-1 α ,25(OH)₂D₃ was 3.65 ± 0.31 (full-term decidua) and 1.03 ± 0.61 (young decidua), and the percentage of tritium recovered as ³H-24,25(OH)₂D₃ was 3.12 ± 0.46 and 3.83 ± 0.41 , respectively. Amniotic tissue did not synthesise either 1 α ,25(OH)₂D₃ or 24,25(OH)₂D₃.

The identity of the putative 24,25(OH)₂D₃ and 1 α ,25(OH)₂D₃ was established when samples from the fractions

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eluting from the Sephadex LH-20 columns in the positions of $24,25(\text{OH})_2\text{D}_3$ and of $1\alpha,25(\text{OH})_2\text{D}_3$ were applied to a high pressure liquid chromatography system (Fig. 2). The identity of the putative $24,25(\text{OH})_2\text{D}_3$ was further defined by subjecting the $24,25(\text{OH})_2\text{D}_3$ region to the periodate cleavage reaction¹³, which resulted in the loss of radioactive label for the metabolite. In one experiment, $0.3 \mu\text{g}$ $[1,2\text{-}^3\text{H},4\text{-}^{14}\text{C}]25\text{-OH-D}_3$ ($^3\text{H}/^{14}\text{C}$ radioactivity ratio 4.9:1, isolated from plasma of vitamin D-deficient chicks injected with $[1,2\text{-}^3\text{H},4\text{-}^{14}\text{C}]$ vitamin D_3) was incubated with pieces of decidua and the putative $1\alpha,25(\text{OH})_2\text{D}_3$ was confirmed as a 1α -hydroxylated metabolite by calculating the reduction of the $^3\text{H}/^{14}\text{C}$ radioactivity ratio¹⁴ (with a change in $^3\text{H}/^{14}\text{C}$ from 4.9 to 2.7). The *in vitro* synthesis of $1\alpha,25(\text{OH})_2\text{D}_3$ by human decidua suggests that in pregnancy the kidney is not the only site of 1α -hydroxylation of 25-OH-D_3 . In addition, the present study demonstrates that an extra-renal 24 -hydroxylase activity exists in human placenta and decidua.

The findings reported here could be relevant to the understanding of the maternal-fetal interrelationships of vitamin D metabolism. For instance, the placenta could be a major source of the $24,25(\text{OH})_2\text{D}_3$ accumulating in the fetus when maternal kidneys are programmed to synthesise $1\alpha,25(\text{OH})_2\text{D}_3$. Another possible biological role for the 24 -hydroxylase present in the placenta could be the 24 -hydroxylation of $1\alpha,25(\text{OH})_2\text{D}_3$, in a process similar to that which may occur in the rat intestine⁶. The 24 -hydroxylation of $1\alpha,25(\text{OH})_2\text{D}_3$, an important step in the inactivation of this metabolite^{4,13}, could explain the observation

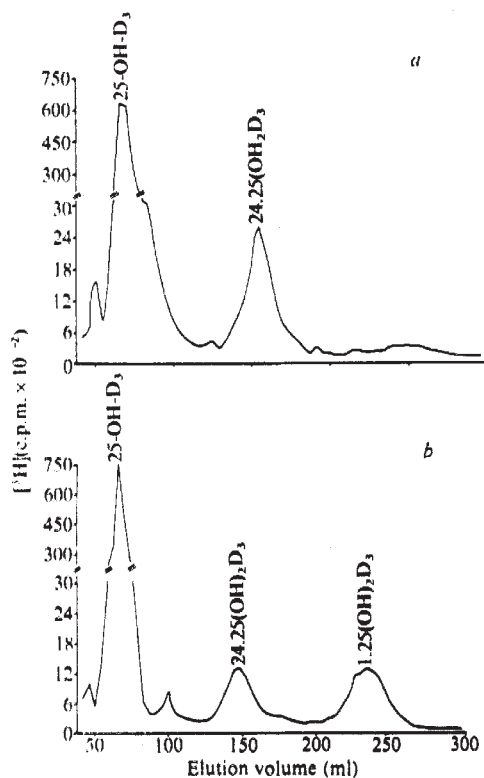


Fig. 1 Characteristic Sephadex LH-20 column chromatographic profile of extracts of full-term human placental tissue (a) and full-term human decidua (b) incubated with tritiated 25-OH-D_3 . Pieces ($2 \times 2 \text{ mm}$) of the tissues were prepared for culture as previously described^{10,11}, pooled to an average wet weight of 300 mg and cultured in incubation flasks with 15 ml of RPMI 1640 medium at 37°C in an atmosphere of 95% air and 5% CO_2 for 48 h, with medium changes every 24 h. At 48 h, 10 pmol $[26,27\text{-}^3\text{H}]25\text{-OH-D}_3$ (Amersham, specific activity 9 Ci mmol^{-1}) in 0.01 ml ethanol were added to each flask. The tissue and medium were extracted at 72 h with chloroform-methanol¹². The lipid extracts were chromatographed on a Sephadex-LH 20 column ($60 \times 1 \text{ cm}$), calibrated with authentic tritiated metabolites and developed with chloroform-petroleum ether (65:35, v/v).

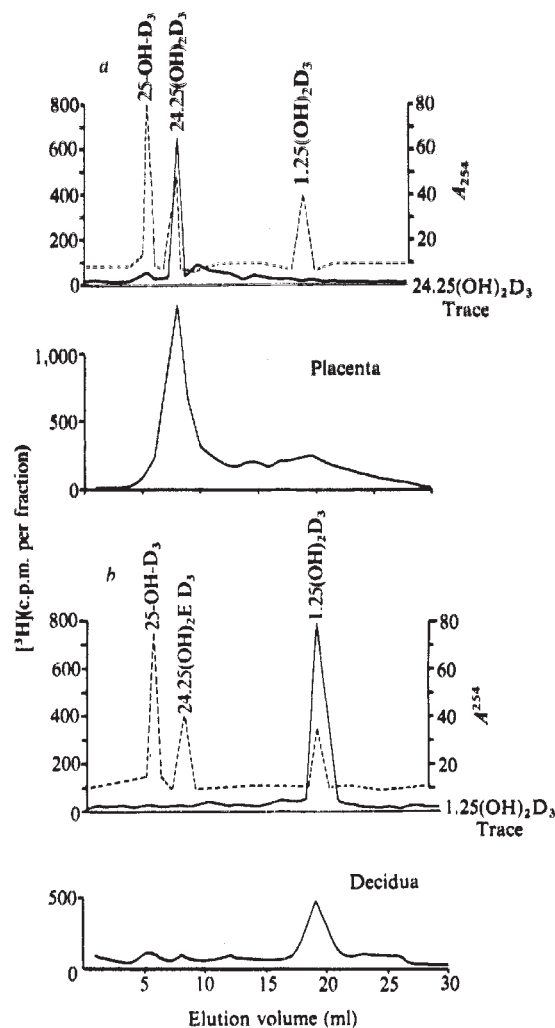


Fig. 2 a, High pressure liquid chromatography of the putative (^3H) $24,25(\text{OH})_2\text{D}_3$ derived from Sephadex LH-20 columns, synthetic $24,25(\text{OH})_2\text{D}_3$ (----) and biosynthesised ^3H - $24,25(\text{OH})_2\text{D}_3$ (—). Extracts of placental tissue incubated for 24 h with $[26,27\text{-}^3\text{H}]25\text{-OH-D}_3$ were chromatographed initially on Sephadex LH-20 (Fig. 1) and the region corresponding to $24,25(\text{OH})_2\text{D}_3$ was then applied to high pressure liquid chromatography (a Waters Associates system fitted with $\mu\text{Porasil}$ columns and eluted with isopropanol-hexane (1:9) with a flow rate of 1.0 ml min^{-1} at a pressure of 280 p.s.i.). A peak of radioactive material coinciding with standard $24,25(\text{OH})_2\text{D}_3$ and biosynthesised ^3H - $24,25(\text{OH})_2\text{D}_3$ is present. b, High pressure liquid chromatography of the putative (^3H) $1\alpha,25(\text{OH})_2\text{D}_3$ derived from Sephadex LH-20 columns (Fig. 1b), synthetic $1\alpha,25(\text{OH})_2\text{D}_3$ (---) and biosynthesised ^3H - $1\alpha,25(\text{OH})_2\text{D}_3$ (—). Experimental details are as in a. A peak of radioactive material coinciding with standard $1\alpha,25(\text{OH})_2\text{D}_3$ and biosynthesised ^3H - $1\alpha,25(\text{OH})_2\text{D}_3$ is present.

that $1\alpha,25(\text{OH})_2\text{D}_3$ is undetectable or present only in small amounts in fetal tissues.

Similarly, the roles of $1\alpha,25(\text{OH})_2\text{D}_3$ synthesised by the decidua remain unclear. One possibility is that it contributes to the observed increase in $1\alpha,25(\text{OH})_2\text{D}_3$ levels in the maternal circulation during pregnancy⁶. Alternatively, $1\alpha,25(\text{OH})_2\text{D}_3$ synthesised by the decidua may be directly involved in regulating the active placental transport of calcium from the maternal circulation to the fetal compartment. A recent report demonstrating the presence of a placental calcium-binding protein similar to rat intestinal $1\alpha,25(\text{OH})_2\text{D}_3$ -dependent calcium-binding protein supports this assumption¹⁵.

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The continuity of thick filaments between sarcomeres in honey bee flight muscle

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One of the most frequently discussed problems of insect flight muscle morphology is the structure of the thick filaments, especially at the Z line. Many attempts have been made to solve these problems but no unequivocal answers have been given so far. It is well known that physiological specialisation is accompanied by certain anatomical features in the myofibrillar level; the myofibrils have very short I bands in the resting state and the muscle acts under nearly isometric conditions. In this paper, a fresh approach is used to demonstrate the fine structure at the Z line in honey bee flight muscle.

The appearance of the thick filaments and the Z line in insect flight muscles is quite different from that in vertebrate muscles. According to many authors^{1–9} there are connections between the thick filaments and the Z line in stretched fibres. In common with all arthropod muscles the Z line consists of a hexagonal network^{1,3,10–13} instead of a tetragonal one. The connections between the thick filaments and the Z line were termed 'connecting' filaments¹⁴.

It is very probable that the connecting filaments not only extend to the Z line but also participate in its structure¹². Consistent with this assumption, it is claimed that there is a continuity of the thick filaments from one sarcomere to the next across the thick filaments prolongations within the Z line^{9,13,15,16}. The term 'thick filament' is used here in a structural sense and does not imply that the entire structure contains myosin. On the other hand, no evidence was found for the presence of connecting filaments in unstretched fibres^{10,11,17}.

Conflicting results have been obtained by electron microscopy of intact muscles using traditional methods (see refs 18 and 19 which present opposite conclusions in the same insect (water bug). Therefore, attempts are now being made to answer these questions using a different technique.

The study of the native isolated filaments can give valuable results for the elucidation of fine structure. Therefore filaments from the honey bee (*Apis mellifera*) flight muscle were isolated according to the method of Morimoto and Harrington²⁰ with a slight modification. The fresh dorsoventral flight muscles were slightly stretched and soaked in a low ionic strength medium consisting of 5 mM Tris-HCl and 10 mM dithiothreitol, pH 7.8 at 2 °C. After 1 d the muscle was homogenised and centrifuged at 1,000 g for 10 min. Then the sediment was resuspended in low ionic strength solution. This procedure was repeated for 7 consecutive days. This period was sufficient to extract the M line material completely and the Z line material partially. Further-

more, an unusual gap or split²¹ occurred in the centre of the A band at the level of the former M lines. The cause of the split is unknown, but thick filaments seemed to be completely several in this region.

Finally the myofibrillar mass was centrifuged and resuspended in relaxing medium containing 0.1 M KCl, 5 mM MgCl₂, 1 mM EGTA, 5 mM ATP, 20 mM potassium phosphate at pH 7.0. This suspension was homogenised three times for 20 s in a Waring blender. The intact fibrils were centrifuged at 3,000 g for 15 min; the turbid supernatant contained isolated filaments. The thick filaments were separated from the thin filaments by zone sedimentation in a density gradient. The supporting solution was the relaxing medium and a linear gradient of glycerol from 10% at the top to 30% at the bottom of the centrifuge tube was used. A 4-ml aliquot of the filament suspension was layered in each tube. This system was centrifuged in a Spinco SW-27 swinging bucket rotor at 22,000 r.p.m. for 2 h. Six equal volume fractions were collected from each tube, and their biochemical composition was studied by SDS-polyacrylamide-gel electrophoresis²². The first two fractions contained a high amount of myosin and a low amount of 'unidentified protein' (perhaps α -actinin, Fig. 1). The third fraction was almost free of protein. The fourth and fifth fractions contained a little myosin, but a high amount of actin. In the sixth fraction distinct and characteristic bands of myosin, paramyosin and actin were found.

A drop of filament solution was applied to a grid coated with carbon film and was negatively stained with 1% uranyl acetate. The first two fractions contained thick filaments. During intense homogenisation, the thick filaments were broken at the split of the former M lines but in every case connections remained at the level of the former Z lines between the very regularly tapered ends of two different thick filaments from opposite sarcomeres (Fig. 2). On several occasions the tapered end of a thick filament bifurcated at the level of the former Z line and joined with two different thick filaments from the adjacent sarcomere (Fig. 3). These intra-Z portions of thick filaments belong to the basic network of the Z line, they pass from one I-Z junction to the next at a slight angle joining the ends of two thick filaments from opposite sarcomeres. This finding correlates with earlier observations of the angling of the Z filaments in longitudinal sections^{2,12,23}. Biochemical and structural evidence strongly suggests that this network does not contain actin. It is very probable that the 'unidentified protein' (perhaps α -actinin) can play an important role in the composition of this network, for it

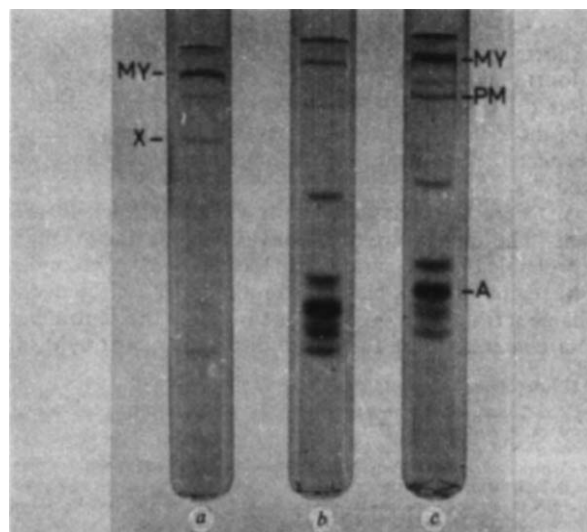


Fig. 1 Gel electrophoresis (10% gel) of sequential fractions obtained from zone sedimentation experiments; (a, second fraction; b, fourth fraction; c, sixth fraction). The bands that were identified by comparison with isolated proteins are marked as follows: A, actin; MY, myosin; PM, paramyosin; X, unidentified protein.

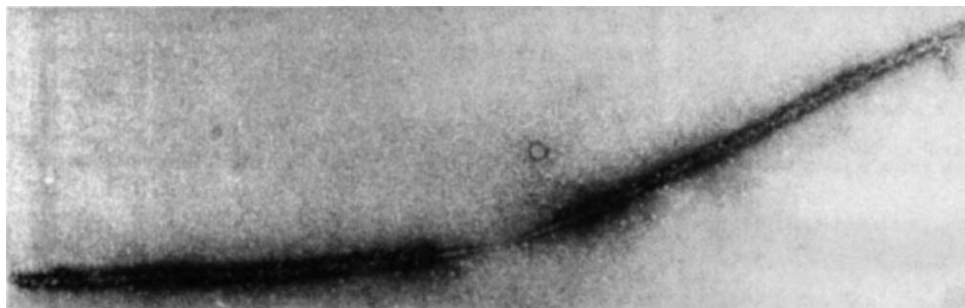


Fig. 2 The very regular tapered ends of two different thick filaments from adjacent sarcomeres, showing them to be connected ($\times 100,000$).

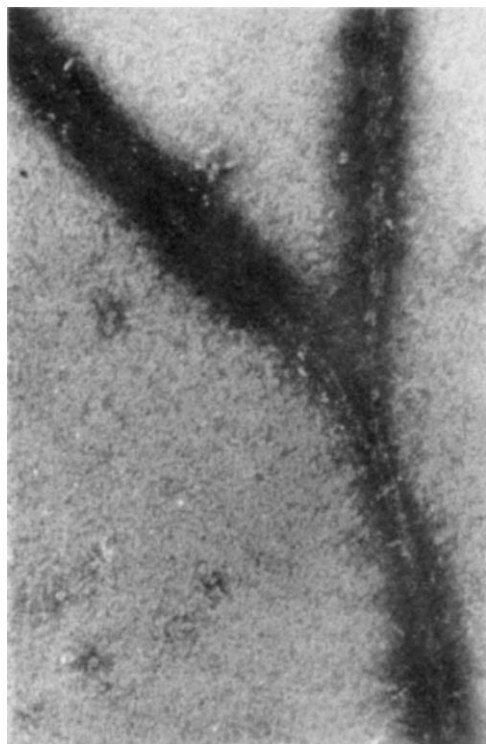


Fig. 3 A single thick filament bifurcated at the level of a Z line and joined with two different thick filaments from the opposite sarcomere ($\times 300,000$).

is well known that the flight muscle Z line contains α -actinin^{21,24}.

The fourth and sixth fractions contained thin filaments and a few short thick filament fragments. According to Ashhurst's model of the Z line^{10,11,18} thin filaments 1.3 μm long or shorter were expected. However, much longer thin filaments were observed.

The sixth fraction contained only thin and thick filament fragments. The appearance of paramyosin in this fraction makes it very probable that the paramyosin localisation²⁵⁻²⁷ is confined to the M region of thick filaments in the bee muscle, since the thick filament fragments originate mainly from the central parts of the sarcomeres where they were disrupted near the M line.

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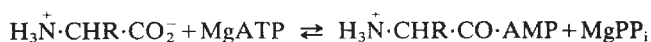
The stereochemical course of amino acid activation by methionyl- and tyrosyl-tRNA synthetases

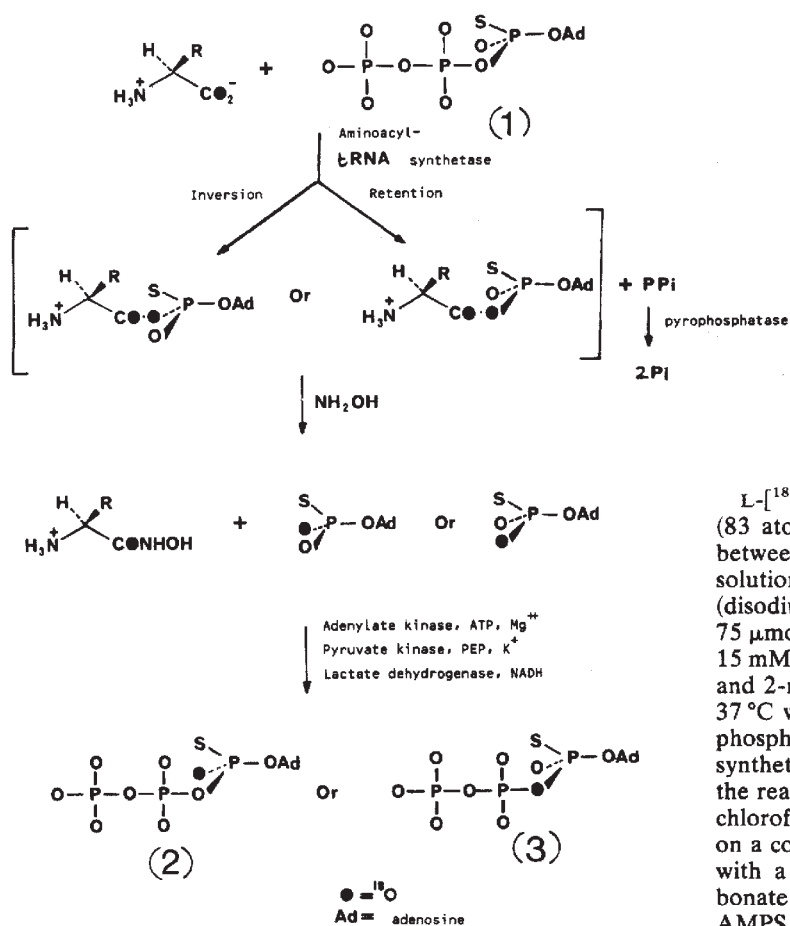
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Stereochemical analysis has long been recognised as a powerful tool for elucidating the mechanisms of chemical and enzyme-catalysed reactions. Although much is known about the stereochemical course of reactions at saturated carbon, phosphate and thiophosphate esters whose ligands to phosphorus are also tetrahedrally disposed, are capable in principle of revealing stereochemical information about events at the active site of enzymes that transform such substrates. Nucleotidyl transferases are a group of enzymes which in general selectively use one of the diastereoisomers of a nucleoside 5'(1-thiotriphosphate), such as isomers A and B of adenosine 5'(1-thiotriphosphate), designated ATP α S-A and ATP α S-B, and allow investigation of the stereochemical course of nucleotidyl transfer¹⁻³. We have developed a simple method based on ³¹P nuclear magnetic resonance spectroscopy for determining the stereochemical course of these reactions, and using this method show here that the nucleotidyl transfer step in two aminoacyl-tRNA synthetases from *Escherichia coli* occurs with inversion of configuration at phosphorus. These observations greatly constrain the mechanistic possibilities for these enzymes, and are interpreted most simply as a direct 'in line' transfer from ATP to the amino acid.

Aminoacyl-tRNA synthetases catalyse two distinct reactions, namely, amino acid activation by MgATP with formation of a mixed anhydride between the amino acid and AMP, and the transfer of the aminoacyl moiety of the aminoacyladenylate so formed to the cognate tRNA⁴.





The amino acid activation step involves substitution at P_α in ATP, and study of the stereochemical course of this reaction should provide insight into the mechanism of this step as well as the geometrical relationship of the substrates at the active site of the enzyme. Thus, observation of net retention of configuration would be consistent with the intermediary of a nucleotidyl enzyme whose formation and breakdown follow the same stereochemical course, or an adjacent attack by the amino acid at P_α of ATP, followed by a single pseudorotation. Alternatively, net inversion of configuration would be interpreted most simply as a direct 'in-line' displacement.

If ATPαS-A (1) (Fig. 1), which is now known to possess the *S*-configuration at P_α (refs 3, 5), is an acceptable substrate for an aminoacyl-tRNA synthetase, incubation in the presence of the cognate [¹⁸O₂]-amino acid and a high concentration of hydroxylamine should lead to the irreversible formation of the amino ¹⁸O-hydroxamic acid and adenosine 5' [¹⁸O]phosphorothioate (¹⁸O-AMPS). If nucleotidyl transfer occurs with inversion of configuration at P_α, the *pro-S* oxygen would be labelled, whereas retention of configuration at P_α would place ¹⁸O in the *pro-R* position (Fig. 1). Conversion of adenosine 5'-phosphorothioate (AMPS) into ATPαS-A by the combined action of adenylate kinase and pyruvate kinase involves phosphorylation of the *pro-R* oxygen^{3,5}. Thus, if ¹⁸O is present in the *pro-S* position in ¹⁸O-AMPS, ¹⁸O will be found in the non-bridging position at P_α in ATPαS-A (2) (Fig. 1), whereas if ¹⁸O is present in the *pro-R* position in ¹⁸O-AMPS, it will be found in the P_α-O-P_β bridge in ATPαS-A (3) (Fig. 1). These alternative possibilities can be differentiated by ³¹P NMR spectroscopy⁵, as ¹⁸O in the P_α-O-P_β bridge produces an isotope shift on both P_α and P_β, whereas ¹⁸O in the non-bridging position causes an isotope shift at P_α only.

Fig. 1 In an aminoacyl t-RNA synthetase-catalysed reaction between the cognate [¹⁸O₂]-amino acid and ATPαS-A (1) in the presence of hydroxylamine, inversion of configuration at P_α leads to adenosine 5' [¹⁸O]phosphorothioate and retention of configuration leads to adenosine 5' [¹⁸O]phosphorothioate. These enantiomeric ¹⁸O-phosphorothioates can be distinguished after conversion enzymatically to ATPαS-A (refs 6, 7), by determining whether ¹⁸O is in the non-bridging (2) or bridging position (3), using ³¹P NMR spectroscopy.

L-[¹⁸O₂]methionine (88 atom % ¹⁸O) and L-[¹⁸O₂]tyrosine (83 atom % ¹⁸O) were prepared by acid-catalysed exchange between the L-amino acid and [¹⁸O]-H₂O (99 atom % ¹⁸O). A solution of L-[¹⁸O₂]methionine (16 mg, 110 μmol), ATPαS-A (disodium salt, 42 mg, 75 μmol), magnesium acetate (17 mg, 75 μmol), Tris (242 mg, 2 mmol), hydroxylamine solution (4 ml, 15 mM, 60 mmol), phenylmethanesulphonyl fluoride (100 mg) and 2-mercaptoethanol (0.2 ml) in deaerated water (60 ml) at 37 °C was adjusted to pH 8.0 with 1 M HCl. Inorganic pyrophosphatase (0.5 mg) was added, followed by methionyl-tRNA synthetase (4 mg). The solution was kept at 37 °C for 48 h and the reaction terminated by addition of EDTA (120 μmol) and chloroform (10 ml). The aqueous phase was chromatographed on a column of DEAE-Sephadex A-25 (HCO₃⁻ form) at 4 °C, with a linear gradient of aqueous triethylammonium bicarbonate buffer (0.2–0.7 M, pH 8.0). Fractions containing ¹⁸O-AMPS (54 μmol, this contained some unlabelled AMPS derived mainly from the hydrolysis of ATPαS-A during the incubation) were pooled and the buffer removed. It was converted to ¹⁸O-ATPαS-A enzymatically^{6,7}. P_α of the ¹⁸O-ATPαS-A showed an isotope shift of 1.1 Hz (at 36.43 MHz) to higher field, where P_β showed none, demonstrating that the ¹⁸O was in the non-bridging position at P_α.

Similarly, when L-[¹⁸O₂]tyrosine (23 mg, 110 μmol) replaced L-[¹⁸O₂]methionine and tyrosyl-tRNA synthetase (4 mg) replaced methionyl-tRNA synthetase, ¹⁸O-AMPS (43 μmol, this contained some unlabelled AMPS derived mainly from the hydrolysis of ATPαS-A during the incubation) was obtained and converted enzymatically^{6,7} into ¹⁸O-ATPαS-A. Again, P_α showed an isotope shift of 1.1 Hz (at 36.43 MHz) whereas P_β was unaffected, demonstrating that the ¹⁸O was in the non-bridging position at P_α.

These observations show that the nucleotidyl transfer step catalysed by both methionyl- and tyrosyl-tRNA synthetase from *E. coli* occur with inversion of configuration at phosphorus.

The simplest interpretation of these results is that nucleotidyl transfer catalysed by these enzymes occurs by an in-line mechanism at P_α. Although more complex multi-step pathways (for example, three steps, each with inversion of configuration, or an adjacent attack with permutational isomerism) cannot be rigorously excluded, it seems unlikely that such mechanisms could occur in the confines of the active site of an enzyme.

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reviews

Survey of medical history

Sydney Selwyn

Explorers of the Body. By Steven Lehrer. Pp. 463. (Doubleday: New York, 1979.) \$12.95.

POPULAR accounts of medical history are surprisingly scarce. Before the appearance of Dr Lehrer's book, the only notable example of this rare genre was *Microbe Hunters*, which was first published over 50 years ago. The author, Paul de Kruif, was a lapsed bacteriologist whose highly coloured descriptions of heroic battles against infection fired the imagination of a wide audience. Indeed, many impressionable adolescents were inspired by his book to enter medicine and related professions.

Sadly, de Kruif's sequels on nutrition, psychiatry and other medical themes were progressively — perhaps predictably — less successful; and although he can be regarded as the main originator of the racy, home-spun style of medical journalism, which remains so popular in North America, it is puzzling that so few writers have attempted to emulate him with full length work in one of the most fascinating branches of human history. Since the 1920s an intermittent series of unexciting biographies has only occasionally been relieved by something as imaginative as *Rats, Lice and History*, written by another bacteriologist, Hans Zinsser. So barren and featureless has been the landscape, that this whimsical little book of 1935 still remains outstanding.

The publication of a reasonably comprehensive survey of medical history for the general reader is therefore to be cordially welcomed. Written attractively and with authority by a practising physician, this new book builds up a mosaic of the lives and work of innumerable contributors to the development of medical science. Four central chapters out of ten are devoted to infection and its control, the remainder being idiosyncratically arranged, starting with physiology, moving to genetics and then anaesthesia. Surgery is embedded among the infections, and the book ends with X-rays and diabetes mellitus.

The opening chapter on the evolution of knowledge about the functioning of the body forms a very satisfying prelude to this vast epic. From the stumbling observations made in Ancient Greece and Rome on the action of the heart and on respiration, progress is clearly traced through the late Renaissance to the work of William Harvey and a succession of other pioneers. Many of them were not primarily interested in medicine, for example, Boyle, Hooke,

Priestley and Black in Britain, and Lavoisier and Laplace in pre-revolutionary France. As an incidental feature, the often uncomfortable (and occasionally fatal) effects of politics on the lives of medical explorers are curiously evident in this introductory section.

Similarly, in the two subsequent chapters fresh insight is provided into such well rehearsed stories as the work of Mendel, the elucidation of the structure of DNA, the acrimonious and complex background to the introduction of anaesthetic gases, and the rise and fall of mesmerism. Although inevitably told with less verve than in de Kruif's pages, Dr Lehrer's accounts of microbe hunting and taming make compelling reading. Particularly vivid are his descriptions of vaccines, antimicrobial drugs and, indeed most other medical advances.

A special feature of the book is its very revealing portraiture of major and minor participants alike. While most are necessarily depicted with the economy of a miniaturist, so extensive yet compact a gallery is unprecedented. Even the cognoscenti will probably realise for the first time just how disagreeable the average medical pioneer is. For every saintly figure such as William Withering or Paul Ehrlich, the author delineates dozens of irascible, bombastic, unscrupulous, or at the least, devious individuals such as Paracelsus, Hooke, Priestley, Erasmus Darwin, Semmelweis, Mendel, Pasteur, Koch, Cushing, Behring, Ross, and a handful of more recent Nobel laureates.

Sometimes, however, debunking seems to be indulged in for its own sake. For example, Harvey is said to have "lacked the ability of a skilled experimenter"

because he considerably underestimated the body's total blood volume (which even now is difficult to measure). Edward Jenner is also criticised on the rather tenuous grounds that he did not publish details about the death of one of his patients, who seems to have succumbed to an unrelated "infection". A further criticism is that Jenner regarded cowpox and a comparable infection of horses as being closely related both to one another and to human smallpox — which indeed is in accord with modern virological views. But after the uncritical recent apotheosis of Harvey during the celebrations of his 400th anniversary, and the sustained adulation of Jenner during the past 150 years, fair re-evaluation of their work is very welcome. The text contains relatively few typographical or factual errors, and solecisms such as the statement that the French Academy of Sciences was, in the eighteenth century, "the final authority in European medicine" are mercifully rare.

The reader may well be disappointed to find virtually no mention of pathology, pharmacology and several medical specialties, including psychiatry — the "Explorers of the Body" being zealously prevented from entering the domain of the mind. An account of some of these important subjects could well have replaced the largely non-medical section on genetics. Nevertheless, the author has accomplished a *tour de force*, and his publishers also deserve praise for producing this admirable book at so reasonable a price. □

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What world food crisis?

Nutrition and the World Food Crisis. By Mary Alice Caliendo Pp.368. (Collier-Macmillan: London, 1979.) £5.95.

ONE function of the applied scientist is to warn his fellow man of possible impending doom, but there is a very real danger of loss of credibility if "Wolf!" is cried too often. There has been a spate of books forecasting food shortage and mass starvation over the past fifty years (two hundred if one counts Malthus), but although the situation is bad it has not noticeably become worse: food production per head of population has

remained almost constant. Indeed, there has been a growing realisation that there is more than enough food about and that the real problem is that the poor cannot afford to buy their daily bread.

Food requirements are about 2,000 kilocalories per day per man, woman and child, and global agriculture produces something in excess of 4,500 kilocalories per head per day of crops suitable for human consumption. Much of this food is used inefficiently for the feeding of livestock to provide animal products for the rich, but even so there is about 2,500

kilocalories per head per day available for human consumption. So, please let us not hear about the 'World Food Crisis'.

All the evidence suggests that farmers can cope with the supply problem providing it is made worth their while. Malnutrition is not a supply problem: essentially economic demand does not equal physiological need. Very roughly half the world is underfed and the other half overfed; and it can be shown that the diseases of affluence — for example, obesity, heart disease, dental caries — are numerically of the same order as those associated with undernutrition. This poor distribution of wealth between nations is of course exacerbated by a further poor distribution within nations, which can lead to local famines. But even in the oft cited Ethiopian famine there was no shortage of food in the country as a whole: the rains failed in one area and the subsistence farmers there did not have enough money to buy food from the market in Addis Ababa.

Nevertheless, the International Agencies constantly document the wide extent of malnutrition in the world and proffer much advice on what to do about it. Not surprisingly this advice is not always consistent: experts often see things from their own narrow viewpoint, and generalists who can grasp technical, economic, social, cultural and political aspects of a problem are rare and often under-rated. Dr Caliendo has made a bold attempt to gather the relevant material, especially that since the 1974 World Food Conference. But sadly the inconsistencies have not been analysed critically or even pointed out. The need to increase food production is stressed in one chapter, but the next on distribution begins with the statement that production is about twice that needed. In chapter 2 the misplaced emphasis in the past on the role of protein in the aetiology of malnutrition is revealed, but in chapter 12 protein-rich foods are promoted. The importance of socio-economic factors is stressed in chapter 10 but their relevance to infant feeding practices in chapter 9 is not adequately discussed: bottle feeding is adopted by many intelligent mothers because it enables them to go out to work and thus improve the economic (and nutritional) status of their families. The current fashion for national food and nutrition planning is outlined but the fact that developed countries eliminated undernutrition without it is not mentioned, nor are we given one example that it has worked anywhere. Food aid is documented without definition nor mention that it is rarely food and seldom used to feed hungry people. These few examples are a true reflection of the inconsistency of the documents available, but perhaps it would be strange if such a complex problem as world hunger were to have a single simple solution.

Many modern texts are collections of papers by various authors often from

Symposia and have the disadvantage that the information is not integrated as in good old-fashioned textbooks. Dr Caliendo has sadly not grasped the opportunity to do this but tries to be all things to all men. It is difficult to see where she stands herself. The book is aimed to "provide educators and students with substantive and practical information" with a view to "make it easier to learn about the problems of global hunger and nutrition". There is no doubt that it is a most useful compendium of

information but because it is so uncritical I suspect it will confuse all but the brightest student. However, the book is cheap, extremely well illustrated with photographs, tables and graphs, and a jolly good read.

D.S. Miller

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Einstein's influence on politics and philosophy

Albert Einstein: His Influence on Physics, Philosophy and Politics. Edited by P. C. Aichelberg and R. U. Sexl. Pp. 220. (Friedr. Vieweg: Wiesbaden, Germany, 1979.) DM48.

I NEVER really liked C.P. Snow's theory of the Two Cultures. In the United States of the 1950s and 1960s it seemed to me that scientists had a good grasp of politics and that liberal arts graduates were genuinely interested in and capable of understanding the main lines of scientific development. But perhaps the only way to understand the durability of the myth of Einstein as a socially naive genius is to accept Snow's basic assertion — there exist two cultures, literary and scientific, whose members cannot talk to each other. On the science side, Einstein's physics continues to mystify the general educated public. And on the arts side Einstein's politics remain incomprehensible to scientists who invariably characterise him as being naive when they write about his life.

The present volume of essays, commissioned by the International Society on General Relativity to celebrate Einstein's centenary, misses its chance to balance the picture. Editors Aichelberg and Sexl state in their introduction that the collection demonstrates the "far reaching influence of Einstein's work and his personality on physics, philosophy, theory of science and on the politics of our time". I believe that such a demonstration is possible. But this collection fails in its attempt to present a comprehensive view of Einstein's influence because much of the physics reported will be understood only by specialists and because Einstein's politics and philosophy receive the usual banal characterisation. The editors re-express the old cliché. Einstein's philosophical directness is "naive" and "this naivete is also characteristic for Einstein's involvement towards politics in which he was increasingly involved in his later

years". The summary is wrong on both counts and it is time to do better.

Einstein was involved in politics from at least 1914 onwards and his political actions throughout his life reflected a highly sophisticated socialist's understanding of what was possible for a man in his position to do at any time. Beginning with the courageous anti-War manifesto in Berlin in 1914 which attacked the extreme national chauvinism of War-time Germany, and ending with his support for Julius and Ethel Rosenberg executed in the US for conspiracy to commit treason in the McCarthyite hysteria of the 1950s, Einstein intervened consistently against militarism, racism and fascism. And though his actions have seemed quixotic to close colleagues such as Max Born, they were informed by a detailed socialist analysis of the workings of class society.

It is possible to indicate only briefly the depth of Einstein's approach. Here is his summary of an analysis called "Why Socialism" that he wrote in 1949 for the influential US magazine, *Monthly Review*:

"The situation prevailing in an economy based on the private ownership of capital is thus characterised by two main principles: first, means of production (capital) are privately owned and the owners dispose of them as they see fit; second, the labour contract is free. Of course there is no such thing as a pure capitalist society in this sense. In particular it should be noted that the workers, through long and bitter political struggles have succeeded in securing a somewhat improved form of the 'free labour contract' for certain categories of workers. But taken as a whole, the present day economy does not differ much from 'pure' capitalism".

Einstein was clear about the solution to the problems that this social arrangement causes:

"I am convinced that there is only one way to eliminate these grave evils, namely through the establishment of a socialist economy accompanied by an educational system which would be oriented towards social goals".

These are not the views of a neophyte. Far from being naive, Einstein's radicalism contrasts favourably with the results achieved by the inept liberal nationalism of the German scientific elite. Professor Carl-Friedrich von Weizsäcker's piece on Einstein's politics illustrates the gap between the

approaches. For von Weizsäcker it was Einstein's "aloofness" not his greater political understanding that permitted Einstein to act politically while others, as von Weizsäcker puts it, "conceded — apparently or truly — to believe in the political prejudices of the time in order to function concretely in their midst".

Similarly Banesh Hoffman presents only a superficial view of Einstein's Zionism by failing to situate Einstein's support for the state of Israel in the context of the intense anti-semitism of Europe between the Wars. As Einstein said in 1938:

"Rarely since the conquest of Jerusalem by Titus has the Jewish community experienced a period of greater oppression than prevails at the present time".

Einstein supported the Zionist cause pragmatically and I think it is a mistake to present him as a narrow nationalist. This was his appraisal of the situation in 1930:

"I saw how schools, comic papers and innumerable other forces of the Gentile majority undermined the confidence of even the best of my fellow-Jews and felt that this could not be allowed to continue. Then I realised that only a common enterprise dear to the heart of Jews all over the world could restore this people to health".

But in spite of the fact that the

collection does not live up to the promise of its title there is still much to interest the casual reader. Dennis Sciama offers a nice concise review of the current position in cosmology and Roger Penrose delivers an informative and very nearly comprehensible account of black holes. And of continuing interest are the personal reminiscences of Einstein. Walther Gerlach on Einstein and quantum theory and John Wheeler on Einstein's Princeton days are new additions to the Einstein archive. Particularly welcome is the inclusion of Einstein's last lecture which along with the transcript of his answers to the questions give a vivid picture of Einstein in action as a thinker. Finally, Wolfgang Yourgrau's portrait, "Einstein — and the Vanity of Academia" is a fresh and irreverent view of the German academy in the 1920s. Yourgrau may have gone too far in reporting his mother's shocked observation that Einstein had dandruff, but I think Einstein would approve. The idea of a god with dandruff would have amused him immensely.

Joe Schwartz

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Communicative abilities

Studies of Language Thought and Verbal Communication. Edited by R. Rommetveit and R. Blakar. Pp. 466. (Academic: London and New York, 1979.) £22.80.

OVER the past 20 years Rommetveit has been developing a particular approach to the psychology of language, thought and communication. He calls his position a "social-cognitive" one and it contrasts in particular with the psycholinguistic-experimental approach with its focus on single sentences. Rommetveit has always held that such an approach has led to gross simplifications which could never be of use in comprehending the complexities of the mechanisms underlying our communicative abilities.

This book is a collection of articles by Rommetveit and his collaborators in Oslo. It is a mixture of theoretical statements and

experimental articles. The 35 articles are of very mixed value and there is little attempt to link together the varied work which ranges from word recognition to the verbal maintenance of sex rules in Norwegian. The experimental section of 27 articles would have benefited from severe pruning.

There is better value in the theoretical articles (most of which are not easily available in their original places) where Rommetveit draws on a number of linguistic and philosophical traditions in discussing the nature of language and communication and moving over into hermeneutics and social psychology. I didn't always succeed in understanding, and quite frequently disagreed but found myself stimulated agreeably often. However, Rommetveit never achieves the synthesis he aims for.

John Morton

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Vibrations of molecular crystals

Molecular Vibrations in Crystals. By J.C. Decius and R.M. Hexter. Pp. 391 (McGraw-Hill: New York and London, 1979.) £15.60; \$29.50.

MANY textbooks discuss in considerable detail the lattice vibrations of simple

covalent and ionic crystals but this book is unique in dealing with the vibrations of molecular crystals. As these are of the type that most chemical solid-state spectroscopists deal with, this book will be of interest and value to the growing number of scientists who study experimentally the vibrations of molecular crystals using infrared, Raman or neutron spectroscopy or to theoreticians carrying out lattice dynamical calculations on molecular crystals. It is jointly written by two authors

with an international reputation for their contributions to the vibrational spectra of molecular crystals using infrared spectroscopy. In many ways the book is a natural extension of the now classic work on *Molecular Vibrations* by Wilson, Decius and Cross (McGraw-Hill) which deals with isolated molecules.

The original approach used in this book is to emphasise a representation based on internal coordinates and thus to take advantage of molecular integrity where it is appropriate, but to permit its modification by perturbation theory. In the internal coordinate representation the dynamical matrix becomes the familiar FG matrix of Wilson, Decius and Cross. A new method of calculation of this matrix, valid throughout the Brillouin zone is developed in this treatise.

Many chemical spectroscopists are not familiar with the splitting of longitudinal and transverse optical modes; they will therefore find useful the review of this important topic which is treated both from the traditional macroscopic point of view as well as with microscopic theory. This has not previously been so carefully done.

Six different molecular crystals (HCN, CO₂, CHI₃, NaN₃ and NaClO₃) are used as detailed examples to consider infrared and Raman spectra and their selection rules. In all cases, spectra are discussed with respect to crystal splittings and polarisations. The current theories of these splittings are carefully examined, compared and evaluated in molecular as well as in ionic crystals. The expression of intermolecular coupling of vibration in both excitation and Phonon coordinates is contrasted, and the basis and extent of their equivalence is discussed.

The theory of multiphonon spectra is thoroughly reviewed, covering selection rules, intensity distribution and origin in crystal anharmonicity. The Van Hove and Phillips theories of critical points are carefully explained. In one example, Iodoform-calculated phonon dispersion curves are presented and their consequences are compared with experiment.

In the final chapter, the spectra of impure crystals, both ionic and molecular, are discussed, together with a simple introduction to Green's function treatment of isotopically impure crystals.

The authors are to be congratulated on producing a well written definitive work that will appeal to physicists as well as chemists interested in molecular crystals. This book is likely to prove useful as a text for postgraduate courses on infrared and Raman spectra of solids, self-study reading for students starting research in the subject and as a guide to the extensive literature in this field.

G.R. Wilkinson

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Psychosocial evolution

SIR. — In his review of Garland Allen's *T.H. Morgan: The Man and His Science* (see *Nature*, 278, 786-787; 1979), Professor Darlington makes several very cogent points. Perhaps the most illuminating of all are his remarks about the relations between Morgan, Wilson and Bateson, which are especially valuable in view of the fact that he knew all three men. It is therefore a great pity that, simply for the sake of taking a rather poorly aimed pot-shot at Allen's Marxism, Professor Darlington drags in the canard of "Lamarckism and Lysenkoism", alleging that both Morgan and Julian Huxley opened the door to these horrors. I do not have Morgan's *Scientific Basis of Evolution* to hand but what Huxley says on psychosocial evolution in *Evolution: The Modern Synthesis* (Allen and Unwin: London; second edition, 1963, pp. xlv - xlvii) in no way justifies this charge. It might be objected that "psychosocial evolution" is a cumbersome and pretentious phrase for a very simple and obvious phenomenon. Indeed, I would myself prefer simply "cultural evolution", or the phrase that Huxley used elsewhere in a popular essay (*The Uniqueness of Man*; Allen and Unwin: London; 1941): "cumulative tradition". The idea is a commonplace, for which a list of 'sources'

would be neither necessary nor practicable. This is itself sufficient reason for Huxley not having mentioned Morgan in this connexion. To argue that, because of its radically different nature, it should not be regarded as a form of evolution or of heredity would be difficult in view of the dictionary meanings of those words.

A belief in psychosocial evolution is perfectly compatible with the belief that heredity, in the genetical sense, is diamond-hard. For Professor Darlington to pretend otherwise is just as absurd as it is for Arthur Koestler (*The Case of the Midwife Toad*; Hutchinson; London, 1971) to claim that Waddington's work on genetic assimilation was a vindication of Lamarckism. Nobody who approaches either human history or what is going on in the world today with a relatively unprejudiced mind can doubt that psychosocial evolution is both real and important. To create a false link with Lamarckism is merely to hand the argument to the Lamarckians on a plate.

A.G. Cock

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Cock on evolution

DR COCK complains that I have wrongly connected the evolutionary views of Morgan and Huxley. And also wrongly connected them with the theories of Lamarck and Lysenko and with what I called the "froth" of our social sciences today. These ideas, he says, are "commonplace" and it is not "necessary or practicable" to trace them to their roots. I, however, think it necessary to trace all ideas, scientific or political, true or false, to their roots. I have been trying to do so for over fifty years. May I show how it works?

There were two roots in this case. Morgan had used his reputation as the supposed creator of the theory of heredity to advance a false theory of heredity and evolution in man. And Julian Huxley had used his name to repudiate his grandfather's work in *Man's Place in Nature*; he was therefore able to present himself as the Darwinian who had discovered that after all we need not worry: Darwinism did not apply to man.

Take Morgan first. I did not seriously look at his book when he gave it to me in 1933 (I don't think he looked at my book either). But there it stands. It expresses the main fallacies current in human evolutionary thought today. The first fallacy is that "There are in man two processes of inheritance: one through the physical continuity of the germ-cells; and the other through the transmission of the experiences of one generation to the next". The physical inheritance gives you 3:1 ratios and dogmatic truth of interpretation. It applies to bodily characteristics. But (and here is the second fallacy) it does not apply to the brain: "It is the plasticity of man's brain that makes him unique". Then Morgan naturally asks: "Is intelligence inherited?". How can we be sure? There is nature and nurture. And there may be conflict between them. Worse still, there is "the doctrine that all men are born free and equal". All the issues are avoided. All the fallacies are scrambled.

Three years later (in 1936) this kind of loose talk had become dangerous. In Europe genetic assumptions were being turned to political uses. Half-baked genetic ideas were helping us on the way to war. In this country a particular danger concerned me. Genetic teaching and research hardly existed in our universities. I therefore collected university signatures for a letter in *Nature* (138, 972-973; 1936) protesting against academic stagnation in this vital

respect. I also turned to Huxley who had been aggressively defending the Mendelian and Darwinian positions at the British Association. I persuaded him to edit an international symposium on the developing situation in evolutionary theory. He agreed. But by the time the book appeared in 1940 (under the title of *The New Systematics*) I discovered that Huxley was easing out of the genetic problem so far as man was concerned. In *We Europeans*, followed by *The Uniqueness of Man* he was looking for a political way out: the kind of 'placebo' that Morgan had stumbled upon.

Faced with this confusion I tried to make my position clear in an article, or manifesto, under the heading of "Race, Class and Mating in the Evolution of Man" (*Nature*, 152, 315-319; 1943). Here I discussed the complex and often reciprocal or feedback relations of race, culture and selection, as recorded in history but usually misunderstood by historians. I argued that inbreeding always produces groups or communities or races which are the indispensable agents of human evolution.

Each of these "owes its character to what we may call its chromosome pool". I went on to say that "race made culture and language" and that inbreeding led to "easy transmission of culture". In other words the creation and transmission of ideas were both based on hereditary abilities, and both were subject to genetic and selective Mendelian and Darwinian processes.

To justify these *ex-cathedra* statements I began to set out the historic and pre-historic record of human society in genetic terms. My first essay, *The Facts of Life*, I sent to Huxley in proof, as he had sent me the chapter of his *Evolution* dealing with my work. He made prodigious annotations, chiefly urging me to qualify my generalisations or weaken my conclusions. I kept the proof but did not adopt the suggestions.

During the next ten years we exchanged a hundred or so letters in which Huxley was enquiring about my views on genetic and evolutionary questions. At the end of this time (in 1963) he published a second edition of his *Evolution*, with a new introduction. Here he set out his full UNESCO formula. Now all peoples were to be reconciled to one another, prosperously united under the banner of inevitable scientific progress.

Man had entered a "psychosocial phase of

evolution". Change was now "primarily cultural and only secondarily genetic". Each human population had, not my pool of chromosomes, but "a pool of ideas" on which it could draw "for its evolutionary requirements". It was obvious (as I explained in the *Times Literary Supplement* of 20 December, 1974) that in this pastiche Huxley had provided the world with a pool of ideas which would meet the "evolutionary requirements" of the social and political sciences for many years to come. Not that they would know (any more than Dr Cock does) what pool they were drawing them from.

For there was indeed another pool. The Soviet Government had repudiated Lysenko in agriculture. But Marxists throughout the world continued to draw what unity they had from one surviving belief, the belief that theories of evolution by natural selection did not apply to man. Anathema had been pronounced on 'Social Darwinism' and it could never be withdrawn. So abominable was this heresy that it must always be condemned; and it must never be discussed.

For these reasons I thought it worthwhile to write another book, *The Little Universe of Man* (apparently unknown to Dr Cock but reviewed in *Nature*, 277, 247; 1979). Here I argued in elementary terms my genetic view that intelligence is seated in the brain and that the brain is part of the body. Hence men differed in their ability to create culture, to acquire culture and to transmit culture. Not only men and women as individuals, but as families, classes and races, as sects and groups of all kinds, they differed genetically by their genes and chromosomes in their abilities to do these things. They always had differed and always would differ, a situation responsible for the past history, present state and future prospects of mankind.

In writing these things I took the opportunity again of holding up Huxley's views to the ridicule they invite and deserve. I also again returned to expose a minor fallacy of Morgan's which has left a long trail of error behind it. This is the view that all heredity is in the chromosomes and in the nucleus. And that therefore one-egg twins are always identical in heredity. Each step in this argument is false and the conclusion has been repeatedly falsified. But it had become, as I put it (*Nature* 234, 521-525; 1971) "axiomatic". Because Morgan said it (although he also said intelligence could not be measured) all psychologists trying to measure intelligence have assumed it to be true — and that includes the late Cyril Burt. I therefore pointed out, first, that Galton, who first recognised one-egg twins, never made Morgan's mistake of supposing they were identical; and secondly, that there are two genetic ways, nuclear and non-nuclear, in which we know why they often cannot be identical. The likenesses of one-egg twins, whether measured in mind or body, remarkable as they are, thus always give an under-estimate of the effects of heredity; their differences always give an over-estimate of the effects of environment.

I may now go back to Dr Cock and his sources. I have found that whether in scientific, historical or any other discussion, it is always desirable to look for the sources, examine them, and question them; and each step in the argument that has led from them. And I believe it is because social and political scientists often fail to do so, finding that the knowledge is "commonplace", or the task not within their "frame of reference", or merely, following the eminent examples of Morgan and Huxley, that so much of what flows from their enquiries turns out to be froth.

C.D. Darlington

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